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TITOLO TESI

***LANDSCAPE CHANGE AND FOREST DYNAMICS: MULTI-
SCALE PATTERN ANALYSIS AND BIODIVERSITY ISSUES***

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A Valentina e alla mia famiglia

ABSTRACT

This thesis was conceived as a contribution to the increasingly urgent need to have sound scientific basis and effective tools to understand, monitor and manage the landscape in order to preserve the biological diversity that characterize landscapes. In this work, concepts, theories, methodologies and tested hypothesis, are proper of Landscape Ecology. Landscape Ecology is a young branch of modern ecology that deals with interrelationship between the spatial pattern and ecological processes in a given land mosaic. In particular, this thesis work offers a double contribution: First, it provides new methodologies for the multi-temporal and multi-scale analysis of the landscape and its changes and; second, it applies Landscape Ecology theories and methods to the conservation and the management of landscape biodiversity issues. The thesis work is organized into three sections, which are in turn subdivided into different chapters. The first section attempts to answer the following question: (1) When Are Two Landscapes Significantly Different? Applications in Temperate Forest Landscapes (Chapters 1, 2). The second section focuses on the analysis of forest fragmentation over time in subtropical forests landscapes: Measuring Forest Fragmentation Over Time: Applications Subtropical Forest Landscapes (Chapters 3-5). Finally, the third section deals with some implementations of Landscape Ecology in biological conservation: Applying Landscape Ecology in Biological Conservation (Chapters 6-10).

The first section of the thesis focuses on the development of new methods in order to (i) provide the statistical significance of the observed differences when comparing different landscapes or their changes over time and (ii) analyze the scale dependence of the spatial pattern change. To properly handle the first issue, a new method to test the statistical significance of pattern metric values between two maps was proposed. In particular, the method was based on the use of Neutral Landscape Models (NLMs) able to reproduce a set of real-like (in terms of composition and configuration) simulated landscapes. For each simulated landscape, pattern metrics can be computed and can be used to produce null empirical distributions for each metric. These empirical distributions can be used to determine if, the observed differences in terms of pattern metrics are significant for a given confidence interval. The second goal was achieved by extending the NLMs approach into the spatial domain to analyze the scale-dependence of landscape changes. Both methods were demonstrated in temperate forest landscapes.

The second section deals with the analysis of forest fragmentation in subtropical ecosystems. Also in this case new methods to quantify the spatial pattern of forests over time were proposed. First, the analysis of the spatial pattern change by means of sample-based techniques. By randomly sampling forest cover multi-temporal maps it was possible to statistically quantify and distinguish changes in forest cover from changes in the spatial pattern of forest. Second, the process of forest fragmentation was studied according to the “landscape context analysis” that is, each forest location was described by its context, using indices derived from the landscape that surrounds it, across a range of scales (from local to regional).

These methods each have their own set of advantages and limitations, and yet may work in a complementary manner. Moreover, in this work the usefulness of the proposed techniques was demonstrated in different environments such as temperate and subtropical forests. However, they can be easily extended to other landscapes such as urban landscapes and, in this respect, they could be an interesting field of further researches.

The third section presents a series of study cases where landscape ecology was applied to biodiversity conservation. In particular, five works were presented: the first deals with the relationship between landscape structure and biodiversity in temperate forest landscapes. The second focuses on the impact of windfarms on the foraging habitat of two bat species at landscape level. The third was aimed to the study of the habitat selection by terrestrial tortoise in high conservation value protected areas. The fourth work analyzes short-term variations of high vegetation summit to the global warming. Finally, the fifth study case deals with the spatial estimation of forest age structure on the basis of remote sensing images and yield models.

All the presented contributions deal with landscape ecology. Landscape change is one of the most important drivers of the biodiversity crisis worldwide. In this respect, the present thesis attempted to give an important contribution to the quantification of the landscape spatial pattern and thus to the relationship between spatial pattern and ecological processes (e.g., fragmentation, habitat selection, etc.). Moreover, these findings potentially addressed the urgent need to have useful tools in order to define conservation measures and management plans, which are essential for the protection of natural and semi-natural habitats and their threatened species in a changing world.

PREFAZIONE

La presente tesi è stata ideata come contributo al crescente bisogno di avere solide basi scientifiche e strumenti efficaci per la comprensione, il monitoraggio e la gestione del paesaggio mirati alla conservazione della sua biodiversità. I concetti, le teorie, le metodologie e le ipotesi testate nel presente lavoro, poggiano le loro basi scientifiche nella disciplina dell'Ecologia del Paesaggio (Landscape Ecology).

L'Ecologia del Paesaggio è una branca dell'ecologia, che ha come obiettivo lo studio delle interazioni che intercorrono tra il pattern spaziale ed i processi ecologici in un mosaico territoriale. Possiamo considerare il paesaggio una porzione eterogenea di territorio composta da insiemi di ecosistemi interagenti e che si ripete con struttura riconoscibile, inquadrata in una determinata zona climatica e geomorfologica e con un determinato regime di disturbi (naturale e antropico) (Forman e Godron 1986). L'Ecologia del Paesaggio studia le cause e le conseguenze dell'eterogeneità spaziale del mosaico di ecosistemi presenti in un territorio a diverse scale spaziali e temporali (Turner et al. 2001). Due importanti aspetti distinguono l'Ecologia del Paesaggio dalle altre discipline ecologiche. Primo, l'Ecologia del Paesaggio enfatizza l'importanza della configurazione spaziale sui processi ecologici. In particolare tiene conto sia della abbondanza dei diversi ecosistemi in un mosaico territoriale (ad esempio la copertura delle foreste in un'area), sia della loro distribuzione nello spazio (spatial pattern). Secondo, l'Ecologia del Paesaggio si focalizza su estensioni spaziali che sono più estese di quelle tradizionalmente studiate in ecologia. Vale a dire studia i rapporti tra il pattern spaziale degli ecosistemi ed i processi ecologici in aree vaste. Recentemente Wu (2013) ha presentato lo stato dell'arte dell'Ecologia del Paesaggio individuando 10 argomenti chiave, ovvero: (1) Pattern–process–scale relationships of landscapes; (2) Landscape connectivity and fragmentation; (3) Scale and scaling; (4) Spatial analysis and landscape modeling; (5) Land use and land cover change; (6) Landscape history and legacy effects; (7) Landscape and climate change interactions; (8) Ecosystem services in changing landscapes; (9) Landscape sustainability; and (10) Accuracy assessment and uncertainty analysis.

Alcuni di questi argomenti chiave sono stati approfonditi durante il presente progetto di ricerca che si propone di fornire un doppio contributo: uno nell'ambito delle metodologie per l'analisi multitemporale e multiscale del paesaggio e l'altro nell'ambito dell'applicazione dell'Ecologia del Paesaggio alla conservazione e gestione della biodiversità.

Il presente lavoro di tesi è organizzato in tre sezioni a loro volta articolate in capitoli. La prima sezione risponde alla domanda: (I) When Are Two Landscapes Significantly Different? Applications in Temperate Forest Landscapes (Capitoli 1-2). La seconda sezione focalizza sull'analisi della frammentazione nel tempo e presenta dei casi studio in paesaggi subtropicali: (II) Measuring Forest Fragmentation Over Time: Applications Subtropical Forest Landscapes (Capitoli 3-5). La terza sezione contiene numerose applicazioni dell'ecologia del paesaggio alla conservazione della natura: (III) Applying Landscape Ecology in Biological Conservation (Capitoli 6-10).

Differenti tipologie di paesaggio sono state utilizzate come “laboratorio” per testare teorie, ipotesi e approcci. Particolare enfasi è stata data ai paesaggi forestali poiché, le foreste, hanno avuto un ruolo fondamentale nella storia dello sviluppo della società umana (FAO 2012). Rudel et al. (2005) hanno coniato il termine "Forest Transitions" per indicare il cambiamento dell'estensione delle foreste a seguito dello sviluppo economico, industrializzazione ed urbanizzazione. Questi cambiamenti possono avere effetti importanti sulla fornitura di servizi ecosistemici da parte delle foreste (p.es. servizi di fornitura, regolazione, supporto e culturali) (Rudel et al. 2005). Nel presente contributo,

sono stati presi in considerazione due paesaggi forestali profondamente differenti: foreste temperate dell'Appennino centrale (Sezione I) e foreste subtropicali localizzate nel nord dell'Argentina (foreste del Chaco arido) (Sezione II). Nel corso dei secoli, questi due ecosistemi, hanno subito cambiamenti differenti che ne hanno determinato l'attuale struttura spaziale. In particolare, mentre per le foreste temperate è in atto un processo di aumento della copertura totale (ricolonizzazione), nelle foreste subtropicali è in atto un severo processo di frammentazione.

Nelle prime due sezioni vengono proposti ed implementati nuovi approcci per quantificare ed analizzare il pattern spaziale del paesaggio. Il pattern spaziale o eterogeneità del paesaggio è costituito da due aspetti principali: la composizione e la configurazione (Turner et al. 2001). La composizione del paesaggio è data dal numero e dall'abbondanza relativa delle diverse tipologie di uso e copertura del suolo, mentre, la configurazione, rispecchia la loro struttura spaziale. La quantificazione del pattern spaziale è un aspetto fondamentale dell'ecologia del paesaggio in quanto permette di (i) monitorare e prevedere i cambiamenti temporali in un determinato paesaggio; (ii) confrontare paesaggi diversi e; (iii) analizzare il rapporto tra struttura e processi ecologici (p.es. distribuzione e movimento degli organismi, flusso di materia ed energia, propagazione del disturbo, etc. - Turner et al. 2001; Wu 2013). Uno degli approcci più diffusi per quantificare il pattern spaziale del mosaico consiste nel calcolo di metriche di paesaggio (landscape metrics) su cartografie tematiche (McGarigal 2013). Tuttavia l'applicazione efficace di tale approccio richiede una solida conoscenza dei principali limiti della Landscape Pattern Analysis (LPA) (Li e Wu 2007): (i) difficoltà nell'interpretazione di alcune metriche fortemente correlate o caratterizzate da relazioni non-lineari (p.es. Remmel e Csillag 2003; Neel et al. 2004; Long et al. 2010; Wang et al. 2014); (ii) complessità nell'analisi di alcune metriche caratterizzate da una consistente risposta multiscalare (Saura e Martínez-Millán 2001; Wu et al. 2002; Wu 2004); e (iii) incapacità di descrivere efficacemente la relazione tra pattern spaziale e processi ecologici (Turner et al. 2001); (iv) assenza di un contesto statisticamente robusto all'interno del quale confrontare il pattern spaziale di due paesaggi diversi oppure dei loro cambiamenti nel tempo (Remmel e Csillag 2003; Remmel e Fortin 2013). In particolare nel primo e secondo capitolo della prima sezione si propone lo sviluppo di metodi in grado di (i) fornire un contesto statisticamente robusto all'interno del quale confrontare il pattern spaziale di due paesaggi diversi oppure dei loro cambiamenti nel tempo (Remmel e Csillag 2003; Remmel e Fortin 2013) (Capitoli 1 – 2) e (ii) analizzare la dipendenza scalare del cambiamento del pattern (Saura e Martínez-Millán 2001; Wu et al. 2002; Wu 2004; Gergel 2007) (Capitolo 2).

Per fornire un contesto statisticamente robusto all'interno del quale confrontare due paesaggi, sono necessarie delle repliche, dalle quali derivare stime, valori medi, intervalli di confidenza (Li e Wu 2007). In particolare, nella LPA, repliche del pattern spaziale considerato sono spesso non disponibili. Un strada percorribile per ottenere le suddette repliche (Capitolo 1), è quella di utilizzare modelli di simulazione del paesaggio (e.g. Neutral Landscape Models - Gardner et al. 1987; Saura e Martínez-Millán 2000). In particolare, i modelli, permettono di simulare un set di paesaggi i cui pattern spaziali hanno proprietà statistiche in comune (composizione e configurazione) con i paesaggi reali. Una volta simulate un numero sufficiente di repliche, sulle quali calcolare i diversi indici di pattern, è possibile ottenere distribuzioni empiriche per ogni indice. Tali distribuzioni vengono utilizzate per confrontare due o più paesaggi e determinare se, le differenze osservate in termini di indici di paesaggio, sono significative ad un determinato livello di confidenza. Nel Capitolo 2 viene adoperato un approccio simile al fine di determinare l'esistenza di scale spaziali significative (Wu et al. 2002), alle quali emergono determinati processi ecologici (p.es. ricolonizzazione naturale dei boschi *sensu* Sitzia et al. 2010). I risultati hanno messo in evidenza che i diversi processi di trasformazione del paesaggio (naturali o antropiche) si manifestano a scale spaziali specifiche.

Per approfondimenti vedere:

Capitolo 1

FRATE L., CARRANZA M.L. 2013. Quantifying landscape-scale patterns of temperate forests over time by means of Neutral Simulation Models. *ISPRS International Journal of Geo-Information* 2 (1): 94-109. doi:10.3390/ijgi2010094.

Capitolo 2

FRATE L., SAURA S., MINOTTI M., DI MARTINO P., GIANCOLA G., CARRANZA M.L. 2014. Quantifying forest spatial pattern trends at multiple extents: an approach to detect significant changes at different scales. *Remote Sensing* 6: 9298-9315. doi:10.3390/rs6109298

Un'altra possibilità per svolgere confronti tra paesaggi in un contesto statisticamente robusto, consiste nell'impiego di un approccio di tipo campionario, grazie al quale derivare stimatori dei parametri di composizione e configurazione e i relativi intervalli fiduciali (Hassett et al. 2012; Stehman 2013). Nella seconda sezione, si adopera un approccio di tipo campionario per analizzare la frammentazione dei boschi nel tempo. La frammentazione è un processo che avviene a scala di paesaggio e consta di due componenti principali: la perdita di habitat (diminuzione della copertura) ed il cambiamento nella configurazione spaziale (pattern spaziale) (McGarigal et al. 2005). Esiste una vasta gamma di metriche di paesaggio in grado di quantificare la dimensione, la forma e l'isolamento delle patches di habitat in un paesaggio (p.es. McGarigal e Marks 1995), ma molte di esse sono fortemente correlate con la copertura dell'habitat (Neel et al. 2004). Confondere il cambiamento nel pattern spaziale con la perdita di habitat può portare a conclusioni erranee e rende impossibile capire quale dei due aspetti è coinvolto nel processo di trasformazione di un paesaggio (Long et al. 2010; Wang et al. 2014). Per rispondere a tale quesito, nei capitoli 3 e 4 è stato proposto un metodo per lo studio della frammentazione delle foreste subtropicali che si basa sulla stima della copertura e configurazione spaziale delle foreste attraverso campionamento di cartografie derivate da immagini satellitari. Tramite tale applicazione è possibile ottenere una valutazione statistica sul contributo relativo della perdita delle foreste (forest loss) e del cambiamento della loro struttura spaziale (spatial pattern change) nel processo di frammentazione. In particolare, è stato adottato l'approccio proposto da Long et al. (2010), che prevede la costruzione di specifici spazi di relazione (relationship space) all'interno dei quali è possibile valutare in che misura la trasformazione del paesaggio è dovuto alla perdita del bosco oppure al cambiamento del pattern spaziale. Inoltre, nel capitolo 4, le possibili ricadute di queste trasformazioni sulla biodiversità degli ecosistemi subtropicali è stata particolarmente enfatizzata.

Per approfondimenti vedere:

Capitolo 3:

CARRANZA M.L., FRATE L., ACOSTA A.T.R., HOYOS L., RICOTTA C., CABIDO M. 2014. Measuring forest fragmentation using multitemporal remotely sensed data: three decades of change in the dry Chaco *European Journal of Remote Sensing* 47. doi: 0.572/EuJRS2044746

Capitolo 4:

CARRANZA M.L., HOYOS L., FRATE L., ACOSTA A.T.R, CABIDO M. Measuring forest fragmentation using multitemporal forest cover maps: forest loss and spatial pattern analysis in the Gran Chaco, Central Argentina. *Landscape and Urban Planning*, under review.

I capitoli sinora presentati considerano il paesaggio secondo il paradigma Patch-Mosaic (Forman 1985) nel quale il paesaggio è un mosaico formato da elementi discreti (patches, matrici, corridoi). Nel capitolo 5, si è scelto di svolgere delle indagini in base ad un approccio complementare al precedente che tiene conto del contesto territoriale nel quale ogni elemento del paesaggio si trova (Landscape Context – e.g McGarigal and Cushman 2005). In particolare, si è scelto di estendere l'approccio della "context analysis multiscalar" (Riitters et al. 2000) all'analisi della frammentazione delle foreste nel tempo. Secondo questo approccio la qualità, la funzionalità e la persistenza degli elementi del paesaggio (p.es., pixel di bosco nel caso di cartografia raster, ecc.) sono fortemente condizionate dalle caratteristiche del mosaico territoriale che li circonda. In altre parole, ogni località classificata come bosco, viene definita sulla base del tipo di frammentazione che caratterizza il paesaggio circostante (p.es. copertura e connettività). Quindi la frammentazione non è più vista come una proprietà stessa del bosco (p.es. dimensione e forma delle patches) ma come una proprietà del paesaggio che contiene bosco. Nella "context analysis" la scelta dell'estensione dell'area indagata ricopre un ruolo essenziale perchè i valori che le metriche di "context" assumono, dipendono dall'estensione del paesaggio indagato (extent). In questo capitolo, vengono analizzate le trasformazioni temporali in termini di copertura e connettività delle foreste subtropicali, a scale multiple (context di dimensioni diverse) che variano da scale locali (~ 5 ha) a scale regionali (~ 8000 ha) (*sensu* Riitters and Wickham 2012). I risultati mettono in evidenza la presenza di processi di frammentazione sia a scala locale che a scala regionale. Di conseguenza grandi distese di foresta sono diventate molto rare oppure completamente scomparse probabilmente come risposta ai cambiamenti nelle modalità di sfruttamento delle risorse imposti negli ultimi 30 anni da differenti livelli di organizzazione antropica (dallo sfruttamento locale dell'ambiente fino alla conversione completa di intere foreste che avviene a scale regionale e nazionale).

Per approfondimenti vedere:

Capitolo 5

FRATE L., CARRANZA M.L., HOYOS L., ACOSTA A.T.R , CABIDO M. Temporal changes in forest spatial pattern at multiple extents: Patch, perforated, edge and interior forests in the Gran Chaco, Central Argentina. *Ecosystems*, submitted.

Da quanto descritto finora emerge che il paesaggio è un elemento dinamico in quanto soggetto a cambiamenti nello spazio e nel tempo e questi cambiamenti si verificano a scale differenti. I cambiamenti nella composizione e struttura del paesaggio hanno serie conseguenze sulla diversità biologica degli ecosistemi naturali e semi-naturali (Wiens 2002) ed, in particolare, il cambiamento della copertura e uso del suolo costituisce la più grande minaccia alla perdita della biodiversità a livello globale (Foley et al. 2005). Alterazioni nella struttura, composizione e funzione del paesaggio possono influenzare notevolmente la dimensione della popolazione (e popolamento), la loro distribuzione, le loro dinamiche (dispersione, movimento per la ricerca di cibo) e la loro persistenza (Gutzwiller 2002). In questo senso, *L'Ecologia del Paesaggio*, offre le basi teoriche, i concetti e gli strumenti necessari per studiare, monitorare e pianificare il territorio in relazione alle problematiche di conservazione della biodiversità (Wiens 2002). Proprio sulla base di queste considerazioni e di quelle effettuate nelle sezioni precedenti, la terza sezione è stata costruita come una raccolta di casi

studio in cui, gli strumenti dell'Ecologia del Paesaggio, vengono applicati alla studio e conservazione di habitat e specie particolarmente minacciati. Nel Capitolo 6 vengono studiati gli effetti della frammentazione sulla biodiversità di flora vascolare in foreste temperate. In particolare vengono analizzate le relazioni tra il pattern spaziale (dimensione, geometria e grado di isolamento delle patches) di un habitat forestale tipico dei paesaggi appenninici italiani (Faggeti degli Appennini con *Taxus* e *Ilex* – EU cod. 9210*) e le caratteristiche di tali formazioni, in termini di biodiversità (composizione di flora vascolare e ricchezza di specie), struttura (Forme biologiche), strategie adattative (specie ruderali, di margine e specie di bosco - interior specialists) ed ecologia (bioindicatori di Ellenberg). I risultati confermano l'esistenza di un rapporto tra frammentazione e biodiversità dei boschi. In particolare le macchie grandi e ben connesse tendono ad ospitare un maggior numero di specie diagnostiche dell'habitat d'interesse comunitario (EU cod. 9210*) mentre nei boschi di piccola estensione sono presenti un numero elevato di specie di margine. Inoltre, gli indicatori strutturali ed ecologici hanno messo in evidenza una qualità ambientale superiore nelle macchie di foresta estese.

Per approfondimenti vedere:

Capitolo 6

CARRANZA M.L., FRATE L., PAURA B. 2012. Structure, ecology and plant richness patterns in beech forests. *Plant Ecology and Diversity* 5: 541-551. doi: 10.1080/17550874.2012.740509

Nel capitolo 7, si affronta la problematica della conservazione di specie volatrici in relazione alla presenza di impianti eolici sul territorio. Attraverso la costruzione di modelli di distribuzione (SDM – p.es., Franklin 2013) per due specie di chirotteri (*Nyctalus leisleri* e *Pipistrellus pipistrellus*) sono state costruite delle cartografie dell'habitat di foraggiamento ottimale delle specie. L'analisi della distribuzione degli impianti eolici in relazione all'habitat delle specie ha messo in evidenza l'esistenza di aree critiche per la loro conservazione. La presenza di fattorie eoliche costituisce un'importante minaccia per le specie indagate in quanto porta ad un processo di frammentazione (incisione, dissezione e perforazione) del loro habitat.

Per approfondimenti vedere:

Capitolo 7

ROSCIONI F., RUSSO D., DI FEBBRARO M., FRATE L., CARRANZA M.L., LOY A. 2013. Regional-scale modeling of the cumulative impact of wind farms on bats. *Biodiversity and Conservation*, 22 (8): 1821-1835. doi: 10.1007/s10531-013-0515-3.

Il Capitolo 8, indaga sulla conservazione e la gestione degli habitat di duna costiera tenendo conto delle loro valenze sia in termini di biodiversità floristica che di fauna. Nello specifico vengono analizzate cartografia digitale di dettaglio relativa agli habitat d'interesse conservazionistico (Direttiva Habitat 92/437CEE) con dati radiotelemetrici di *Testudo hermanni* come esempio di specie di fauna minacciata (EN IUCN 2014) e caratteristica di questi habitat. L'analisi dell'"habitat preference" (Manly et al. 2002) ha messo in evidenza una stagionalità nella scelta delle tipologia di vegetazione da parte della *Testudo* di Hermann che risponde alle diverse esigenze trofiche, di termoregolazione e riproduttive durante l'anno.

Per approfondimenti vedere:

Capitolo 8

BERARDO F., CARRANZA M.L., FRATE L., STANISCI A., LOY A. Seasonal habitat preference by the flagship species *Testudo hermanni* and implications for the conservation of coastal dune EU habitats. *Journal for Nature Conservation*, submitted.

Nel capitolo 9, si analizza la risposta del paesaggio vegetale di alta quota al riscaldamento climatico globale. Vengono studiate le trasformazioni della vegetazione sommitale avvenuta durante gli ultimi 10 anni sulle vette italiane (Appennino centrale e Alpi Sudorientali - rete LTER). I risultati mostrano che i paesaggi di alta quota tendono ad arricchirsi di specie provenienti da piani vegetazionali inferiori (termofilizzazione) e caratterizzate da strategie di riproduzione vegetativa (emicriptofite e camefite).

Per approfondimenti vedere:

Capitolo 9

STANISCI A., FRATE L., MORRA DI CELLA U., PELINO G., PETEY M., SINISCALCO C., CARRANZA M.L. 2014. Short-term signals of climate change in Italian summit vegetation: observations at two GLORIA sites. *Plant Biosystems*. doi: 10.1080/11263504.2014.968232

Nel capitolo 10 viene proposta e testata una procedura per la cartografia dell'età dei boschi in base a immagini telerilevate e modelli alsometrici. Nella regione Molise, i boschi si presentano come un mosaico dinamico composto da patches riferibili a diversi stadi di successione ecologica (Gustafson e Diaz 2002). Alcune tipologie forestali, come i boschi di roverella presentano valori di età piuttosto bassi (meno di 30 anni), mentre i boschi di faggio e cerro presentano una distribuzione per classi di età più ampia e generalmente sono caratterizzate da stadi seriali più maturi (dai 10 a >120 anni).

Per approfondimenti vedere:

Capitolo 10

FRATE L., CARRANZA M.L., DI FEBBRARO M., GARFI V., TONTI D., MARCHETTI M., OTTAVIANO M., CHIRICI G. Spatially explicit estimation of forest age integrating remotely sensed data and inverse yield modeling techniques. *iForest*, submitted.

Nella presente ricerca, sono state affrontate numerose problematiche relative all'analisi delle trasformazioni del pattern spaziale nel tempo e alla conservazione della biodiversità di differenti habitat e specie particolarmente minacciati da fattori ambientali ed antropici.

Per l'analisi delle trasformazioni nel tempo sono state proposte delle procedure e delle nuove metodologie che permettono il confronto tra paesaggi in un contesto statisticamente robusto. Per il confronto statistico sia fatto ricorso sia a repliche derivate da paesaggi simulati sia da campionamento di paesaggi reali. In queste analisi il paesaggio è rappresentato da un mosaico di elementi discreti (Patches, corridoi, matrici, ecc.) secondo il paradigma Patch-Mosaic il quale offre una rappresentazione efficace, relativamente semplice e molto utile per l'analisi di diversi processi ecologici come la ricolonizzazione naturale, la frammentazione e per il monitoraggio dei cambiamenti del pattern nel tempo.

Come prima proposta, è stata presentata una nuova procedura adatta a confrontare statisticamente il pattern spaziale del paesaggio nel tempo che si basa sull'utilizzo di paesaggi simulati attraverso modelli di simulazione neutrale (Neutral Landscape Models). L'approccio proposto si è dimostrato particolarmente efficace in quanto ha permesso di: (i) ottenere un set di paesaggi che simulano in maniera adeguata il pattern spaziale delle foreste e il suo cambiamento nel tempo, direttamente confrontabili con paesaggi reali; (ii) rappresentare le informazioni più rilevanti del processo indagato; (iii) ottenere repliche che sono statisticamente simili ai paesaggi reali e quindi permettendo il loro confronto statistico. Inoltre, l'estensione di tale approccio alla dimensione multiscalare ha: (iv) fornito una più accurata comprensione della relazione esistente tra pattern e processi; e (v) permesso di evitare gli errori potenzialmente introducibili nelle analisi a scale singole che ignorano le informazioni sulla distribuzione e la variabilità delle metriche di pattern.

Tuttavia questo tipo di approccio può presentare dei limiti legati essenzialmente a: (i) la correlazione tra i parametri di pattern (metriche) e la composizione del paesaggio (abbondanza); e (ii) la difficoltà dei modelli di simulazione (NLMs) nel riprodurre alcuni paesaggi dove i fattori ambientali ed antropici generano mosaici con strutture complesse (es. forme lineari e allungate). Per quanto riguarda la prima limitazione, al fine di indagare la relazione tra pattern spaziale e composizione e le rispettive trasformazioni nel tempo, è stata proposta una nuova metodologia che prevede la costruzione di appositi "spazi di relazione". Gli spazi di relazione mettono in confronto i valori che assumono le metriche di pattern e i rispettivi valori di copertura (composizione). La costruzione degli "spazi di relazione" si è rivelata uno strumento efficace per valutare il contributo relativo di composizione e configurazione, al processo di frammentazione nel tempo, specialmente per quelle metriche che presentano relazioni non monotoniche.

Come possibile alternativa per superare le eventuali limitazioni proprie dei NLMs, durante il presente progetto di ricerca è stata testata la possibilità di svolgere il confronto statistico, non più basato su simulazioni di paesaggi ma attraverso stimatori (media, deviazione standard) derivati dal campionamento di paesaggi reali. Il campionamento di serie temporali di cartografia telerilevata ha permesso di (i) ottenere stime non distorte delle metriche di pattern spaziale e del relativo cambiamento nel tempo; (ii) fornire le basi per il monitoraggio costante nel tempo del processo di frammentazione; (iii) valutare le trasformazioni (traiettorie) in uno spazio di relazione che descrive le caratteristiche spaziali (pattern e composizione) specifiche dei territori indagati.

Durante la ricerca si è anche tentato di testare dei metodi di analisi spaziale che tengono conto della gradualità caratteristica di molti processi ecologici e delle sfumature e transizioni che caratterizzano

i paesaggi reali. Tra questi metodi la "context analysis", capace di descrivere la composizione e la connettività del contesto territoriale nel quale si trova ogni elemento di paesaggio, costituisce una valida alternativa al Patch-Mosaic model per l'analisi della frammentazione. In particolare, la context analysis (i) offre una rappresentazione realistica della natura sfumata di numerosi processi ecologici; e (ii) fornisce una misura diretta della scala spaziale alla quale i processi ecologici avvengono (connettività e composizione variano in funzione del paesaggio circostante o extent incluso nell'analisi).

Infine, le ricerche svolte nell'ambito della conservazione della biodiversità di habitat e di specie hanno confermato le grandi potenzialità dell'Ecologia del paesaggio nell'orientare ed ottimizzare le misure necessarie per una gestione sostenibile dei diversi ecosistemi. L'Ecologia del Paesaggio continua ad offrire strumenti efficaci per lo studio e il monitoraggio dell'eterogeneità spaziale, per lo studio della relazione tra pattern e processi e per la conservazione della biodiversità. Si tratta di una disciplina in continua evoluzione. Il presente lavoro di tesi ha confermato le potenzialità dell'Ecologia del Paesaggio ed ha fornito nuovi elementi che contribuiscono a rispondere ad alcune delle sue grandi sfide per il futuro. Ad esempio il contributo alle conoscenze sulle relazioni che intercorrono tra il mosaico spaziale (e quindi la sua quantificazione) e i processi ecologici (p.es. frammentazione, dispersione e movimento di organismi) risponde alla necessità di avere strumenti adeguati per definire misure di conservazione e piani di gestione capaci di migliorare il destino degli habitat naturali e semi-naturali e delle specie che ospitano.

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SEZIONE I.

When Are Two Landscapes Significantly Different? Applications in Temperate Forest Landscapes

CAPITOLO 1

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Article

Quantifying Landscape-Scale Patterns of Temperate Forests over Time by Means of Neutral Simulation Models

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Abstract: Several studies attempt to describe changes in the spatial patterns of forests over time, resorting to the comparison of landscape pattern indices (LPI), but new methods for quantifying landscape differences in a statistical context are necessary. In this paper, we quantified and assessed the statistical significance of the forests pattern changes, which have occurred since the end of WWII in Central Italy (Isernia). To do this; based on the proportion of forest cover (p_i) and contagion (H) of three land cover maps (1954–1981–2006); we generated 100 forest maps with predictable results through the midpoint displacement algorithm. Then, for both observed and simulated maps, we computed a set of LPI (number of patches, cohesion, largest forest patch index and area weighted mean shape index) and we derived their empirical distributions; finally, we compared the empirical distributions using the non-parametric Kruskal-Wallis test. Our results show significant changes in the spatial pattern of forests and underline the process of natural forest re-growth, which, in the area, is constrained by “remnants” of traditional activities. The adopted approach could be extended to a large ensemble of landscapes and spatial scales and could become a standard procedure when comparing patterns in time.

Keywords: midpoint displacement algorithm; landscape pattern index; empirical distribution; forest regrowth

1. Introduction

Landscapes change in both structure and function [1] and said dynamism is mainly driven by changes in management practices responding to social, political and economic forces [2]. The analysis of temporal and spatial changes represents one of the most challenging problems in landscape ecology. Describing changes in different land cover types through time may be crucial, both for preserving biological diversity and related ecosystem services, and for developing general landscape models, which are useful in ecosystem management and environmental policies [3,4]. The current state of landscape patterns in the world is mainly the result of centuries of evolution in land use [5]. For instance, temperate forests in mountain and hilly landscapes of Europe are currently distributed in a mosaic, which is derived not only from centuries of extensive forest exploitation, but also from agriculture and cattle farming [6–8], followed by a recent, spontaneous reforestation process, which occurred after the World War II rural exodus and the consequent abandonment of traditional agricultural practices [9–14].

The quantitative, spatial and temporal analysis of natural forest re-growth in abandoned farmlands has acquired increasing relevance due to the effects of forest expansion on many important ecosystem functions [11]. For example, forest expansion affects hydrological cycles and soil dynamics [15], climate [16,17] and biodiversity [11,18,19] at different scales. Several landscape metrics have been developed to quantify forest patterns in terms of space and time, most of which have been tested on grid-based thematic maps [5,20–22]. Studies aimed at quantifying forest distribution frequently employ landscape pattern indices (LPI) to measure changes in both forest cover and pattern. Changes over time are assessed by many authors simply through the comparison of LPI [23–25]. Current research suggests that the comparison of raw LPI values should be avoided [26], since they are sensitive to scale [27], land cover proportions [28], spatial resolution [29,30], spatial extent [31] and land-cover misclassification [32]. New methods for comparing LPI values may be useful in order to add statistical context to landscape pattern analysis [27,33]. As at present it is possible to clearly identify and quantify the differences between any two given dates, the statistical significance of the observed changes remains the most important and complex challenge with which to deal [28,34]. In order to enrich landscape pattern analysis with statistical significance, new methods for comparing and testing LPI values have been proposed [34–36]. In ecological research, at the landscape scale, replication cannot be considered, and consequently, distribution, expected values and variance are not available; ecologists who want to perform statistically robust comparisons of landscapes among different maps must rely upon simulations based on computer-generated models able to reproduce an expected pattern, which shares statistical properties with an empirical pattern of interest [34,35,37].

Among the spatial models developed in landscape ecology, the neutral models (NLMs) are those able to produce an expected pattern in the absence of specific landscape processes [21,38–42], providing a baseline in order to evaluate the influence of landscape heterogeneity on ecological processes and *vice versa* [42]. The first neutral model in landscape ecology was the simple random map developed from percolation theory [39,43] and created by randomly assigning habitats to a proportion, pi , of a grid map [39]. Subsequently, the evolution of NLMs followed the development of fractal methods applied to percolation maps [42]. The second generation of NLMs were hierarchical, random landscapes, generated by fractal curdling [44]. Both the above-mentioned NLMs assume a

complete spatial independence from the habitat (or cells in a map) throughout a landscape. Since, in real-life landscapes, habitats usually show a certain degree of spatial contagion, another fractal algorithm, specifically the “midpoint displacement algorithm” [45] has been proposed, giving way to a third generation of NLMs, whose surfaces exhibit continuous environmental variability. Successively, many attempts to test their usefulness in various fields of research have been made. The NLMs based on the midpoint displacement algorithm have been used to assess the effect of landscape fragmentation on population distribution [46], dispersal success of communities [47], insect movement patterns [48] and biodiversity [49]. They were also used to model the diffusion of contamination in aquatic environments [38], the influence of correlated spatial patterns on species coexistence of plant communities [50] and the spatial pattern of disturbance [51] at different scales [52,53]. Although, initially, many NLMs were theoretically applied [54], several efforts toward modeling complex landscapes and testing their differences using neutral fractals have been made [35,55,56]; therefore, we are confident that such an approach can be applied to landscape comparison over time, while carrying out a statistical significance assessment.

Considering the aforementioned points, the present work aims to describe forest cover dynamics in the hilly landscapes of Central Italy in the last 60 years, analyzing the spatial pattern of temperate forest patches of the area surrounding a small city in a rural European setting in detail (Isernia municipality). In particular, we focus on two questions:

- (1) How did the spatial pattern of temperate forests change over time (1954–1981–2006)?
- (2) Is the spatial pattern change of these forests significantly different over time?

To properly handle such an issue, on one hand, we used a set of landscape pattern indices to describe the forest spatial pattern dynamics and, on the other hand, we compared LPI of real and simulated landscapes to assess the statistical significance of any possible differences. In order to simulate fractal maps, we chose the midpoint displacement algorithm, because it looks very promising for modeling the natural reforestation process, which occurs in many hilly landscapes, as it is able to represent continuous autocorrelated pattern variations [40]. In particular, this fractal NLM allows, through the variation of both proportion and spatial contagion, the obtaining of different levels of habitat aggregation and patchiness [57]. In order to better understand the dynamics of forest fragments and to offer the basis for pinpointing specific conservation actions and forest management strategies for temperate forests in Europe, a multi-temporal analysis, which takes into account the statistical significance of differences in landscape, should be implemented.

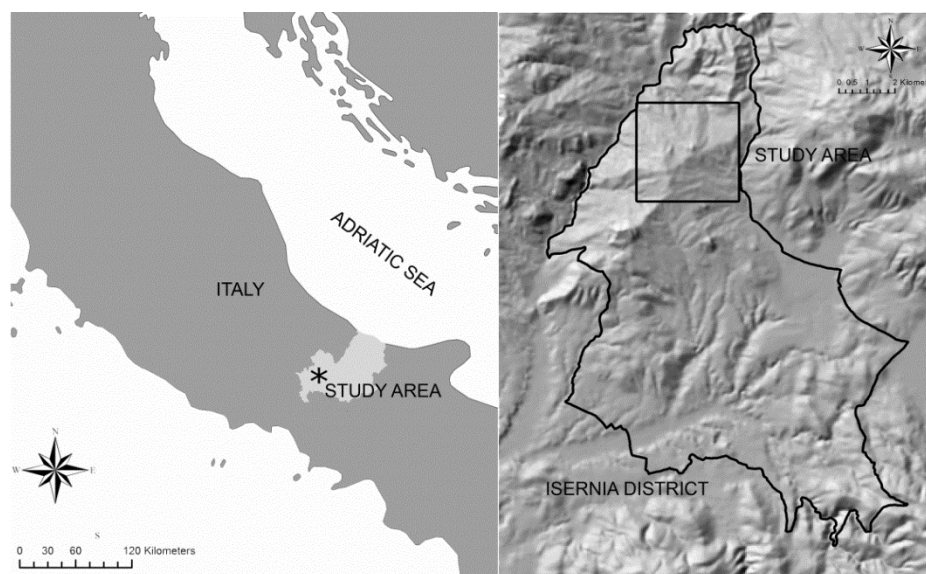
2. Methods

2.1. Study Area

Isernia (Central Italy) was selected for our analysis (Figure 1), because the abandonment of traditional rural activities in this area, along with recent developments in infrastructure, appear to have caused changes in land cover, which are typical of small cities in many areas of Italy and other Mediterranean countries [58]. Furthermore, the municipality of Isernia represents a pilot study area of a larger project aimed at analyzing the landscape dynamics of rural areas, which have occurred since the end of World War II in Central Italy. Isernia covers *ca.* 68 ha and, at present, consists of a small

continuous urban area (22,000 inhabitants) surrounded by a hilly landscape with agricultural, semi-natural and natural land cover types: olive groves, crop fields, complex cultivation patterns and broad-leaved forests (mainly *Quercuspubescens* and *Quercuscerris* woodlands). Although these forests are very important from a conservation point of view (“Pannonian-Balkanic turkey oak-sessile oak forests” according to “Habitat Directive” EEC 43/92), no protected areas exist in Isernia. Altitudes range from 291 m above mean sea level (a.m.s.l.) to 906 m a.m.s.l., and the climate is temperate [12]. During the last half century, almost 50% of the territory has changed, mainly due to an evolution in landscape management trends: agricultural intensification in the alluvial plains and abandonment of traditional land use activities on the reliefs and less accessible areas [58]. In 1955, the study area was characterized by widespread agricultural lands accompanied by natural and semi-natural vegetation patches of broadleaved forests, thickets and pastures. On the contrary, more recently, broad-leaved forest cover increased in less accessible and hilly areas, substituting shrub lands, part of pre-existing grasslands and olive groves [58].

Figure 1. Map showing the Isernia district (Italy) and the location of the study area.



2.2. Data Analysis

We analyzed the spatial pattern of forests and assessed the significance of the observed differences over time, following the general framework proposed by Rempel and Csillag [35], as described in Figure 2.

2.2.1. Data Preparation

Three land cover maps, relative to the years 1954, 1981 [58] and 2006 (1:25,000), derived from aerial photographs, were used to perform the landscape pattern analysis at the regional scale (see Acosta *et al.* [58]).

Due to the fact that the midpoint displacement algorithms can generate only square maps [55], we delineated a representative (approximately 20% of hilly sectors of Isernia) 256 × 256 pixel square area, which was used to extract the same geographical window from each map to be compared in time

(Figure 3). Next, land cover maps were rasterized with a spatial resolution of 10 m and reclassified in two categories: broadleaved oak forests and other cover types (arable land, permanent crops, pastures, shrub and herbaceous vegetation).

Figure 2. Proposed framework for comparing and testing differences of forest pattern over time.

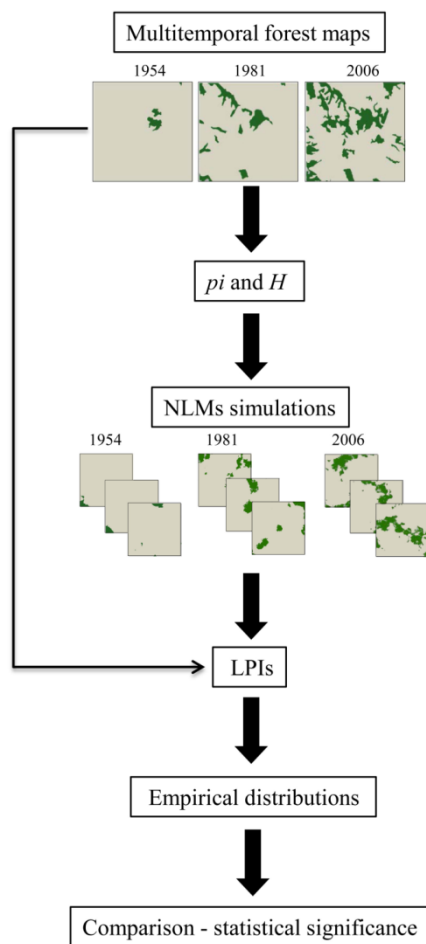
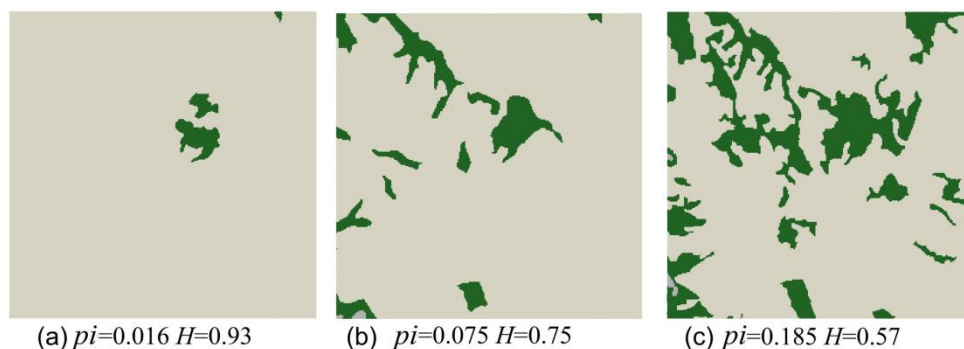


Figure 3. Three forest cover maps extracted from a multitemporal dataset of Isernia, Italy. Each image is 256×256 pixels, with a spatial resolution of 10 m. The binary classification separates oak forest (dark green) from other cover types (arable land, permanent crops, pastures, shrub and herbaceous vegetation—soft grey). pi indicates the proportion of forest cover; H is the autocorrelation of the map. (a) = 1954; (b) = 1981; (c) = 2006.



2.2.2. NLM

Firstly, two parameters necessary for running the NLM simulations were calculated for each date (1954, 1981 and 2006): proportion of forest cover (pi) and contagion (H). pi is given by the number of forested cells in relation with the total number of cells present in the landscape and ranges between 0 (no forest cover) and 1 (whole landscape covered by forests). H , or contagion [59], describes the adjacencies of forest “cells” and ranges from 0, when forest distribution is maximally disaggregated (no adjacencies among cells of the same class), to 100, when landscape is totally covered by forests (only forest-to-forest adjacencies). Next, for each above mentioned map, we generated 100 maps using the public domain software Qrule [57], freely available on-line (<http://www.al.umces.edu/faculty/bobgardner.html>). Fractal landscapes were generated using the midpoint displacement algorithm [45], as described in With [60] and With *et al.* [46]. In short, for each simulation, a three-dimensional fractal surface with roughness controlled by H was created by the midpoint displacement algorithm; then, every fractal surface was sectioned at the appropriate elevation to create a two-dimensional landscape map with the requisite amount of forest given by pi . Finally, in order to avoid the “salt and pepper” artifact commonly present in fractal maps [55] and to make simulated maps comparable with real landscapes [56], we deleted all the patches smaller than the minimum mapping unit of the original maps [61]. The removal of small patches didn’t significantly affect the proportion of forest cover (pi reduction < 0.5%).

2.2.3. LPI Calculation

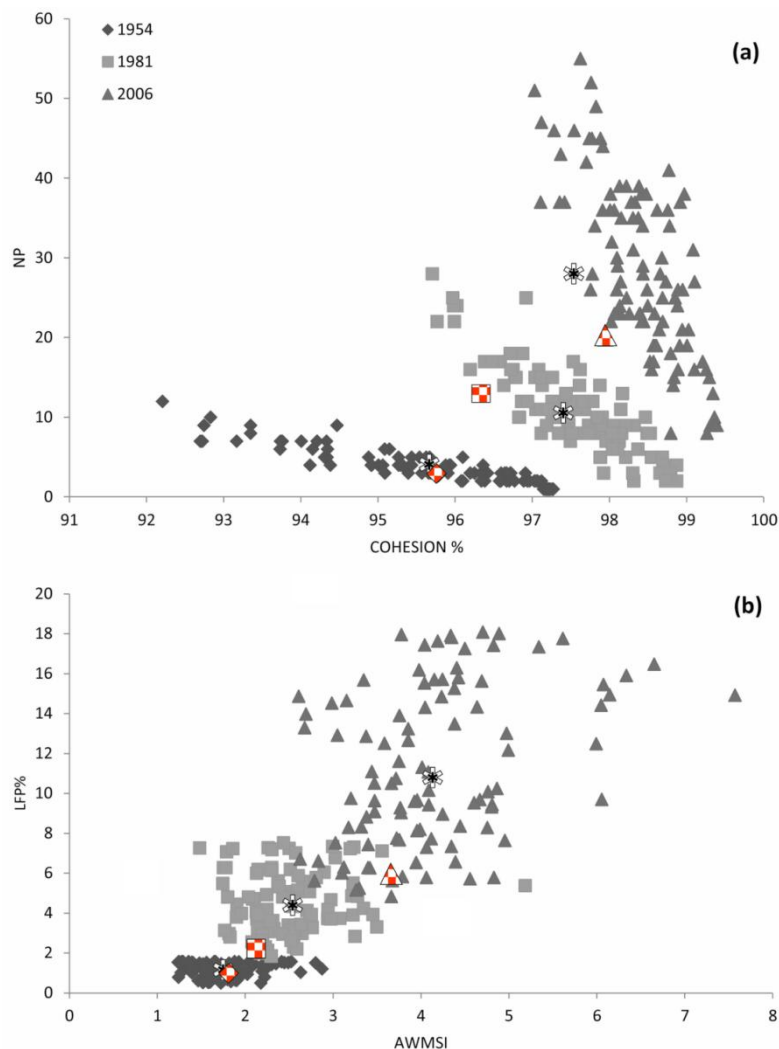
To analyze the spatial pattern of forests through time, a set of landscape pattern indices on both real and simulated maps was calculated by using FRAGSTATS 4.0 [62]. After recording the total number of patches of forest (NP), we focused on the other three class landscape metrics, which had been previously reported as ecologically meaningful [22,63] and have been proven to be useful in describing patch spatial structure in a forested landscape context [64–69]: largest forest patch index (LFP), area weighted mean shape index (AWMSI) and patch cohesion index (COHESION). NP and LFP were selected, because they are related to forest fragmentation [33,66,67,70], defined as the breaking up of one large forest area into many smaller patches [71]. The largest forest patch index (LFP) quantifies the percentage of total landscape area comprised by the largest forest patch. As the diminution of LFP over time is one of the most effective metrics for measuring forest fragmentation [67], its increment could be an effective indicator for describing the reverse process [72,73]. AWMSI [74] measures the complexity of patch shape compared to a standard shape that, in raster format, attains its minimum value (AWMSI = 1) for squares. AWMSI values increase for more irregular and elongated shapes. We chose AWMSI, because of its capacity to distinguish between big, round-shaped patches, characteristic of well-preserved forests, and small, irregular patches, which often dominate in disturbed landscapes (for a review, see Haines-Young and Chopping [64]). COHESION measures the physical connectedness of forests and is commonly used for describing habitat connectivity [75,76]. In conditions of natural forest re-growth, an increment in time of landscape cohesion and connectivity could be expected [69]. COHESION, which ranges between 0 and 100, is minimal when the proportion of the forested landscape decreases and becomes increasingly subdivided and less physically connected. On the other

hand, COHESION increases as the proportion of the landscape covered by forests increases (see McGarigal and Marks [74] for details).

2.2.4. Comparing Observed and Simulated LPI

By computing the selected LPI (NP, LFP, AWSI and COHESION) on simulated landscapes, their empirical distributions (*sensu* Fortin *et al.* [77]) for each date were obtained. First, we verified the ability of the midpoint displacement algorithm to produce plausible patterns and underlying processes by generating scatterplots that include LPI derived from both real and simulated maps (Figure 4). In particular, the following LPI-based two-parameter spaces were built: NP *vs.* COHESION and LFP *vs.* AWMSI. Then, we quantified the differences of LPI among the three dates (1954, 1981 and 2006) by performing a Kruskal-Wallis test, followed by a Mann-Whitney post-hoc pairwise test.

Figure 4. Scatter plots among two paired combinations of observed landscape pattern indices (LPI) values for simulated landscapes (N = 100; 1954, 1981 and 2006): (a) total number of patches of forest (NP) *vs.* COHESION and (b) LFP *vs.* weighted mean shape index (AWMSI). Asterisks indicate the mean values, and red symbols show the position of real landscapes within the empirical distribution derived from simulated landscapes (in grey scale).



3. Results and Discussion

The analysis of the 1954, 1986 and 2006 maps shows consistent changes on both the abundance and spatial distribution of forests (Figure 3). During the last 60 years, the proportion of forests increased from $pi = 0.016$ in 1954 to $pi = 0.185$ in 2006, as the spatial contagion decreased from $H = 0.93$ (1954) to $H = 0.57$ (2006). From the post-war until the end of the analyzed period, we observed the natural spread of forests, a phenomenon that characterizes the large scale dynamics of oak woodlands in many hilly and mountainous landscapes of the Italian peninsula [14,58,68,78]. In Mediterranean areas, the socio-economic post- World War II changes led to a decline of the number of people involved in traditional agricultural and grazing activities [78–82]. Consequently, natural re-growth of forests took place in abandoned lands [58,83].

The rate of forest spread, which in the first time span (1954–1981) was of 5.9%, increased to 11% in the second one (1982–2006) and is in contrast with previous studies, which pointed out an overall decrease in the rate change of secondary successions over time [84–86], because of the occupation of the most suitable sites during the first stages of colonization. In our case, in fact, the slow and gradual cessation of farmland practices, the persistence of agricultural activities [58] and the overexploited conditions of soil in abandoned farms slowed down the establishment and growth of woody vegetation [68,86]. Instead, the observed increment of forest expansion rate after 1981 is probably related to two main factors: (i) the consistent abandonment of agricultural practices occurring in Isernia during this period [58], which provided new areas to be colonized and; (ii) the presence of nuclei of forest regrowth, which ecologically facilitated landscape evolution towards a more natural condition.

In Figure 4, scatter plots showing both the position of real and simulated landscapes within the LPI-based two-parameter space (NP vs. COHESION and LFP vs. AWMSI) are reported. It is important to note that real landscapes (red symbols) fall inside the simulated landscapes scatter cloud (grey symbols). Despite the fact that many authors stress the limits of NLMs to capture the structure of real landscapes [87], the use of the midpoint displacement algorithm has provided a reliable set of simulated maps, which adequately describe the spatial pattern of forests through time (Figure 4). The fact that all the LPI calculated from real landscapes fall within the area of the respective distribution, obtained from simulated landscapes, contrasts with previous works, which state the merely theoretical value of such simulations [42,54]. It is important to note that the midpoint displacement algorithm constitutes the starting point for modeling more realistic and complicated scenarios of landscape change [38,40,60]. In our case, we obtained landscapes with several degrees of patchiness and spatial aggregation, by tuning the parameters that control landscape simulations (pi = proportion of forest cover and H = forest spatial autocorrelation), of the values observed in the different maps. A significant change in all LPI values was observed when analyzing the spatial pattern of forests (Kruskal-Wallis test, $p < 0.01$). In particular, the number of forest patches (NP) significantly increased by about 66% (3–13) from 1954 to 1981 and by about 61% (13–20) from 1981 to 2006, resulting in an overall 87% (3–20) increase for the entire period (1954–2006). The significant rise in the number of patches, caused by the establishment of several new nuclei of young forests into abandoned farmlands [68], characterizes the former stages of natural colonization of abandoned lands in Mediterranean ecosystems [70,78–80,88,89]. Contemporarily, we also detected a significant increment in the percentage of landscape occupied by the largest forest patch: in 1954, the percentage of landscape

occupied by the largest forest patch was 1.17% (S.E. \pm 0.034), while it significantly increased both in 1981 (4.47%, S.E. \pm 0.16) and 2006 (11.26%, S.E. \pm 0.39). This is mainly due to the enlargement, over time, of existing vast nuclei and their coalescence with contiguous ones [14,68,78–80,88–90]. During the advanced phases of spontaneous regeneration in Mediterranean areas, forest cover tends to evolve into a more homogeneous distribution, with a general decrease in the number of patches [14,68,90] derived from the expansion and coalescence of the numerous small pioneering forest patches into a few larger ones. In contrast with the general trend observed inside a natural reserve in Tuscany, where the protection regime allowed for a complete regeneration of forests [68], in the analyzed territory (where no conservation or protection constraints exist), only a partial forest re-growth occurred. Indeed, as evident in other Mediterranean landscapes [83–90], many “remnants” of the traditional activities in Molise hilly landscapes are still present and constrain the distribution and natural spread of forests. Over the three “sampling dates”, the shape of forest patches (AWMSI) significantly increased, ranging from 1.74 (S.E. \pm 0.035) in 1954 to 2.48 (S.E. \pm 0.052) in 1981, with a further increase in 2006 (4.13, S.E. \pm 0.091). In terms of COHESION, there was a significant increase of the physical connection of forest patches. In particular, the COHESION index was 95.69% (S.E. \pm 0.14) in 1954 and increased to 97.54% (S.E. \pm 0.075) in 1981, reaching 97.96% (S.E. \pm 0.056) in 2006. As previously described in Mediterranean landscapes, the re-colonization of oak forests in Molise occurred with an increment over time of shape complexity (AWMSI) [83,91] and connectivity (COHESION) values [78,82]. Immediately after the abandonment of traditional agricultural practices, the fine-grained pattern, which characterizes landscapes affected by long-established agriculture, formed by small regularly shaped and isolated patches, was progressively replaced by a more coarsely grained pattern, which results in large, irregularly shaped forest patches [83,91].

4. Conclusions

The observed increment in extension and the significant changes in spatial distribution of forests suggest that the analyzed area underwent an intense process of natural re-colonization, which has slowly begun after World War II and which is still in progress. The phenomenon we observed could be considered as reforestation (*sensu* Sitzia *et al.* [69]), that is, the natural reestablishment of a forested landscape on disused agricultural lands following farm abandonment [70] in regions where the potential natural vegetation (*sensu* Zerbe [92]) is a forest. The presence of many (NP) irregular patches (AWMSI), with increasing values of connectivity (COHESION) and patch dimension (LFP), underlines a transitional stage of forest re-growth.

Although the accurate description of the huge ecological consequences of such transformations is beyond the scope of this work, we can point out possible effects, such as an increment in true forest species [93,94], a stronger connectivity for forest vertebrates [76,95], an improvement in CO₂ sink services [96], regulation of water drainage [97] and landslide prevention [98].

The NLMs effectively modeled the pattern of forests over time at a specific spatial resolution and could be also very useful for exploring future scenarios, responding in this way to the urgent need to predict natural reforestation process [86]. Simulation models could also help to better understand how natural re-growth varies in space and time and its effects on landscape function [71]. In particular, predicting the pattern of forest expansion is extremely important, because of its possible effects on

many key ecosystem functions [99,100]. For instance, the pattern of forest expansion affects watershed services [15,101,102], biodiversity [11,18,19] and climate at different scales [16,17].

Many of the insights and conclusions obtained in this study have been facilitated by the statistical framework provided by the utilization of NLMs. In particular, the application of the midpoint displacement algorithm:

- (1) allows for modeling a reliable set of maps, which adequately describe the spatial pattern of forests through time and which can be directly compared with real landscapes [56];
- (2) generates a set of landscape replications, which account for the most relevant information;
- (3) defines the landscape expectations, which allows the statistical comparison of patterns through time [29,47,48].

It is important to note that the obtained results are strongly dependent on both the specific type of landscape and the chosen spatio-temporal scale. Since, by tuning the scale of analysis, the observed patterns and the underlying processes become finer or coarser, a sound study of landscape evolution over time should include the spatio-temporal scale [103]. Therefore, such an approach could be extended across a large ensemble of landscapes [37,55] and spatio-temporal scales [52,53], thus providing relevant indications as to the changes in landscape structure over time and all the ecological and cultural consequences linked to this issue [99].

Furthermore, statistical analysis could become a standard method when comparing maps [20], especially in change detection procedures, so that a sound basis for developing efficient management policies of forests could be provided.

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CAPITOLO 2

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Article

Quantifying Forest Spatial Pattern Trends at Multiple Extents: An Approach to Detect Significant Changes at Different Scales

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Abstract: We propose a procedure to detect significant changes in forest spatial patterns and relevant scales. Our approach consists of four sequential steps. First, based on a series of multi-temporal forest maps, a set of geographic windows of increasing extents are extracted. Second, for each extent and date, specific stochastic simulations that replicate real-world spatial pattern characteristics are run. Third, by computing pattern metrics on both simulated and real maps, their empirical distributions and confidence intervals are derived. Finally, multi-temporal scalograms are built for each metric. Based on cover maps (1954, 2011) with a resolution of 10 m we analyze forest pattern changes in a central Apennines (Italy) reserve at multiple spatial extents (128, 256 and 512 pixels). We identify three types of multi-temporal scalograms, depending on pattern metric behaviors, describing different dynamics of natural reforestation process. The statistical distribution and variability of pattern metrics at multiple extents offers a new and powerful tool to

detect forest variations over time. Similar procedures can (i) help to identify significant changes in spatial patterns and provide the bases to relate them to landscape processes; (ii) minimize the bias when comparing pattern metrics at a single extent and (iii) be extended to other landscapes and scales.

Keywords: modified random cluster algorithm; pattern metrics; scalogram; forest regrowth; stochastic simulations; central Italy; statistical significance of change

1. Introduction

Forest ecosystems have played a major role in human history, and periodic deforestation has accompanied population growth and development throughout the world for thousands of years [1]. Although tropical forests are affected by intensive deforestation because of the dependence of local populations on land-based economic activities, harvesting practices, of vital importance in the postwar economy, have recently become moderate in temperate forests [1]. In particular, the current distribution of temperate forests in several hilly and mountainous landscapes of Europe derive from centuries of extensive forest exploitation followed by the abandonment of traditional agricultural practices [2–4]. In this context, the analysis of forest spatial pattern through time and, in particular, the study of the process of natural regrowth is of primary importance in ecological research because forest distributions could affect many ecosystem functions at multiple scales [4].

The most common approach for analyzing changes in forest spatial patterns over time is the mere comparison of pattern metrics extracted from areas of fixed size defined by administrative or natural limits [5,6]. However, such an approach could be problematic for at least three principal issues: (i) the arbitrary choice of the extent of the analyzed area [7]; (ii) the lack of specific scale breaks (thresholds) to identify significant changes in landscape structure and function [8]; and (iii) the negligence, or, in the worst cases, the absence of an analysis of statistical significance when comparing categorical maps [9]. Indeed, forests, like other ecological systems, are characterized by a hierarchical spatial structure [10–14] where specific patterns and processes may take place at certain “characteristic” spatial extents (scale effect) [15]. This means that, for example, different forest dynamics can be most effectively studied at a particular characteristic extent. Thus, identifying this characteristic extent provides a key to further understand the processes that occur in a specific ecological system. It follows that limiting the analysis of forest distribution to a single spatial extent could introduce potential bias or misleading conclusions in pattern analysis [16,17]. Furthermore, the majority of pattern metrics, commonly used to quantify and monitor forest spatial distribution, are scale-dependent, and their scale sensitivity has been demonstrated (see Šimová and Gdulová [7] for a review) for both empirical [18–22] and simulated landscapes [16,18,23]. In fact, the limitations and pitfalls introduced as a result of the use of landscape metrics to compare landscapes with different map sizes are well documented (see Sitzia *et al.* [24] for examples), and there is a critical need for further research addressing the influence of spatial extent on pattern analysis over time. An accurate knowledge of metric scaling relations could be given by empirical scalograms in which variations of pattern metrics are plotted directly against scale [25,26]. In this context, we believe that multi-temporal scalograms could help in

both cases: Relate the observed patterns to underlying ecological processes and correctly extrapolate the recorded information across scales. Finally, although the observed spatial patterns are the realization of specific spatial processes [27], it is of great importance to understand whether the observed differences between two patterns could have arisen purely by chance or whether a specific process has promoted these differences [9]. Nevertheless, the attribution of statistical significance to differences in forest pattern over time is still one of the most important and complex challenges to be faced [27]. The statistical comparison of two different landscapes is quite difficult to perform because field studies usually address only one or a few landscapes so that no simple test is available for making statistical inferences [9,15,27]. One possible way to compare and test the statistical significance of pattern metric values between two maps is the use of computer-generated simulations (e.g., Neutral Landscape Models) to reproduce a set of maps with spatial characteristics (composition and configuration) that are similar to real-world characteristics [27–31]. Among the neutral landscape models [9,31], the Modified Random Cluster Method (MRC) [32] is able to correctly represent forest aggregation (or fragmentation) caused by human land use pressure [33]. By varying simulation parameters (the proportion of forest cover p_i and the degree of aggregation H), it is possible to obtain different levels of habitat aggregation and patchiness [32]. In view of the above, we are strongly confident that such statistical methods, if extended to multi-temporal analysis, could offer a consistent framework for assessing forest pattern changes over time and sound information necessary for relating them to landscape processes.

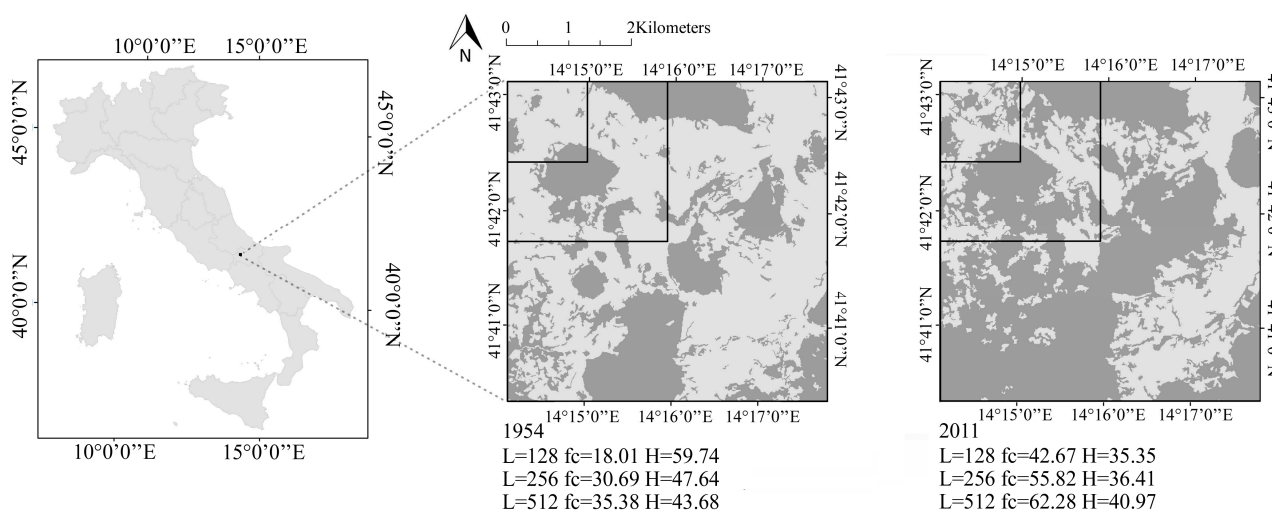
Based on consideration of the aforementioned points, we propose and test a procedure in this study to quantify the spatial pattern of forests over time at multiple spatial extents with statistically robust methods. As an application, we focus on forest cover dynamics in hilly landscapes. We analyze the spatial pattern of temperate forest patches in a Man and Biosphere Reserve (MAB-UNESCO) in central Italy as a representative example of landscape transformation occurring during the past 60 years in sub-Mediterranean hilly landscapes. In particular, we attempt to clarify the following questions by implementing the proposed procedure: (i) how did the forest pattern vary on the compared dates in relationship to various spatial extents? (ii) do specific scale breaks exist that indicate consistent changes in landscape structure and function? (iii) are the differences in the spatial pattern of forests over time statistically significant? To address this issue appropriately, we first used a set of pattern metrics to describe the forest spatial dynamics over time and across various extents and then assessed the statistical significance of any possible differences by comparing the metric values of real and simulated landscapes. An analysis at multiple spatial scales might help to better define the characteristic extents at which it is possible to focus on specific aspects of forest dynamics (e.g., forest loss or gain; forest fragmentation or coalescence of forest patches). The assessment of the statistical significance of forest pattern differences over time and across scales could also offer sound information to relate the observed spatial pattern to the specific underlying ecologic processes and to better understand the specificities of the study case, thus allowing for the application in other cases.

2. Materials and Methods

2.1. Study Area

The Collemeluccio-Montedimezzo Man and Biosphere Reserve (MAB-UNESCO) in Central Italy was selected for analysis (Figure 1). This MAB reserve was chosen because the recent historic changes occurring in this area offer a good example of the pattern of landscape transformation in all sub-Mediterranean hilly landscapes. Since the end of the Second World War, many socio-economic changes have occurred in Europe, where the abandonment of traditional rural activities has produced marked changes in the distribution of temperate forests [34–36]. The reserve and its buffer zone covers approximately 25,000 ha and currently consists of a hilly and mountain landscape dominated by broadleaved natural forests (60% of the area) and other semi-natural vegetation types, such as shrubs and meadows (20% of the area), along with agricultural land and pastures (20% of the area). Altitudes range from 380 m a.s.l., (the Verrino fluvial plain) to 1730 m a.s.l. (Mt. Capraro), and the climate is temperate [37]. The main potential natural vegetation (*sensu* Zerbe [38] and Ricotta *et al.* [39]) is a broadleaved temperate forest [36,37].

Figure 1. Location of the study area, with forest cover maps for the years 1954 and 2011 with a pixel resolution of 10 m. The multi-scale analysis was performed by expanding the extent diagonally, starting from the upper left corner of the original area. The dimension of the maps was 128×128 , 256×256 and 512×512 pixels, corresponding to 163.84, 655.36 and 2621.44 hectares. The binary classification separates forest (dark grey) from no forest (light grey). fc is the proportion of forest cover, and H is the spatial autocorrelation or contagion of the map.



2.2. Forest Cover Maps

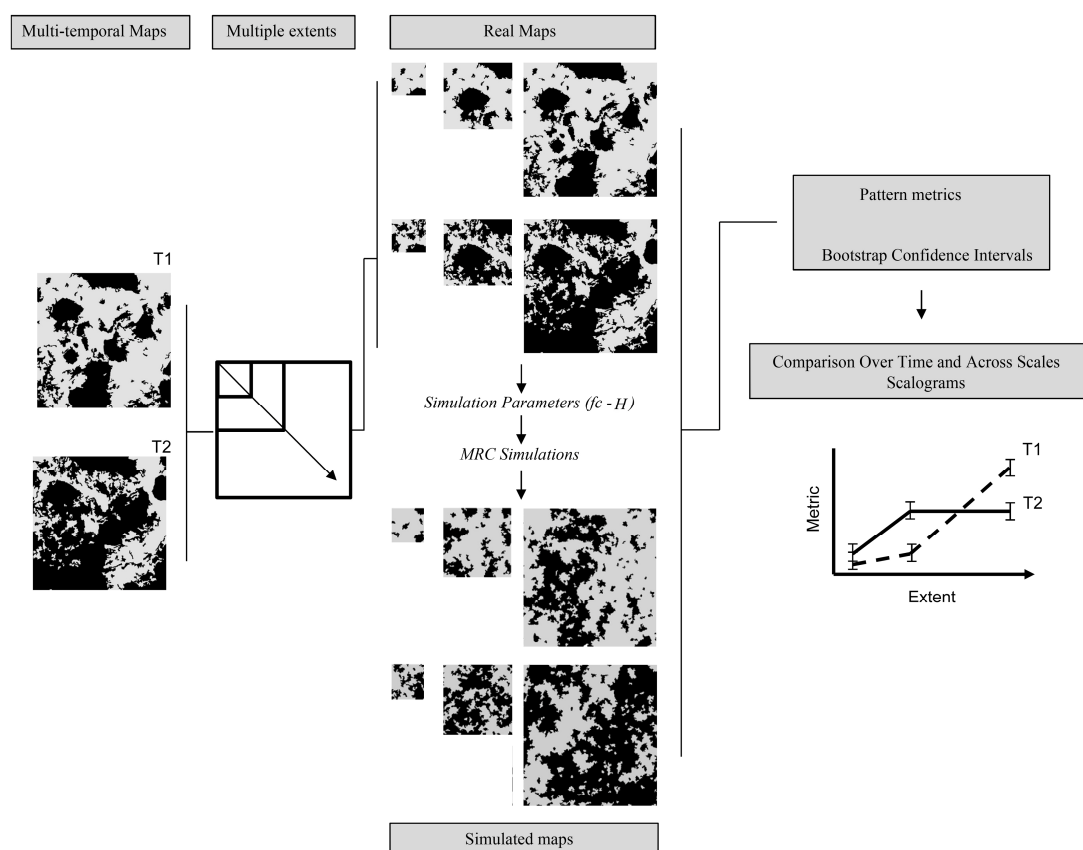
To assess changes in forest distribution, we used existing large-scale (1:8000) forest cover maps of the MAB reserve for the years 1954 and 2011. The 2011 forest cover map was derived by applying a manual classification process (manual segmentation and photointerpretation supported by field data performed in summer 2011) to panchromatic digital orthophotos (flight AGEA05) relative to the

Collemeluccio-Monte di Mezzo Reserve. For the preparation of the 1954 map, a series of greyscale aerial photographs (flight GAI) were acquired, georeferenced and digitized within a Geographic Information System. The 1954 aerial photos were scanned in 8-bit TIFF images with a resolution of 600 dpi and orthorectified using OrthoEngine software (PCI Geomatica) with a 10 m Digital Elevation Model (DEM). For each data frame, 30 ground control points were used for the orthorectification process, and the resulting Root Mean Square Error (RMSE) was less than 4 m. A manual classification process was applied to produce the 1954 forest cover map. Next, both forest cover maps (1954 and 2011) were rasterized with a spatial resolution of 10 m. To make the maps comparable, we set the minimum mapping unit to 0.5 ha by applying a majority filter.

2.3. Data Analysis

We quantified the spatial pattern of forests over time at multiple spatial extents using real and simulated maps and detected significant changes in forest spatial pattern relying on bootstrapping procedures [29] to perform significance testing. The general framework is outlined in Figure 2.

Figure 2. Flowchart representing the different steps of the proposed procedure for detecting significant changes in forest spatial pattern at different scales. T1: 1954; T2: 2011.



2.4. Multi-Scale Analysis

To perform the multi-scale (extent) analysis over time, we selected from both forest maps (1954 and 2011), a set of three representative geographic windows of different dimensions. As suggested by Wu [22], we delineated a first window extent of 128×128 pixels (small) and diagonally expanded it starting

from the upper left corner to the bottom right corner of the original area with the following increasing dimensions: 256×256 (medium) and 512×512 pixels (large). It is important to note that also the window direction can influence the result of a pattern metric analysis, since landscapes are commonly anisotropic [20]. However, we overcome this issue by using neutral simulations (as described below) able to reproduce isotropic landscapes. The analyzed extents correspond to 163.84 ha, 655.36 ha and 2621.44 ha, respectively (Figure 1). The selected window dimensions are comparable with those commonly used for local and regional forest analysis at landscape scale [40]. As suggested by O'Neil *et al.* [19], the chosen extents are at least two times larger than the largest patch area in the year 1954 (see Figure 1). To frame the landscape transformation patterns in an ecologically significant manner, the wider window was entirely included in one homogeneous environmental type [36], *i.e.*, in one potential natural vegetation type [38,39], thus, in an area where in absence of human interventions or hazard events the vegetation would evolve in one potential natural type (see Zerbe [38] and Ricotta *et al.* [39] for details). Note that in our case the analysis on larger windows would include in the extent, areas with different and heterogeneous environmental characteristics (geology, morphology, soils and climate) and, thus, forest dynamics must be interpreted accounting of the presence of extra environmental heterogeneity.

2.5. Spatial Pattern Analysis

To analyze the spatial pattern of forests over time and across extents, we selected a set of eight pattern metrics that had been previously reported as ecologically meaningful and that have proven useful for describing and comparing the spatial structure of forests [41,42]. The selected metrics are adequate for describing forest patch size (MPS = Mean Patch Size), forest subdivision (NP = Number of Patches, PD = Patch Density), forest spatial geometry (LSI = Landscape Shape Index, ED = Edge Density TE = Total Edge), and connectivity (AI = Aggregation Index, CLUMPY = Clumpiness Index). The landscape pattern analysis software FRAGSTATS 4.0 [43] was used to calculate the metrics. The description of the pattern metrics used in the study (based on McGarigal and Marks [44]), along with their respective variation range, are provided in Table 1.

Table 1. List of pattern metrics used in the study. Landscape metrics and the relative acronyms, descriptions, variation ranges and scaling relations based on published references were provided. Symbols: ▲ increase, ▼ decrease, ? unpredictable, = no sensitivity, ▲ ▼ increase then decrease.

Landscape Metrics	Description	Range	Scaling Relation	References
Number of Patches (NP)	The number of forest patches	≥ 1	▲	(Baldwin <i>et al.</i> [21]; Shen <i>et al.</i> [23]; Wu [22])
Patch Density (PD)	The number of forest patches per unit area (patches/ha)	> 0	? ▼	(Shen <i>et al.</i> [23]; Wu [22]) (Saura and Martínez-Millán [16]; Baldwin <i>et al.</i> [21])
Mean Patch Size (MPS)	The average area of all forest patches in the landscape (ha)	> 0	?	(Shen <i>et al.</i> [23]; Wu [22])
Total Edge (TE)	The sum of the lengths of all forest edges in the landscape (m)	≥ 0	▲	(Turner <i>et al.</i> [18]; Baldwin <i>et al.</i> [21]; Shen <i>et al.</i> [23]; Wu [22])

Table 1. Cont.

Landscape Metrics	Description	Range	Scaling Relation	References
Edge Density (ED)	The total length of all forest edges per ha (m/ha)	≥ 0	? =	(Shen <i>et al.</i> [23]; Wu [22]) (Saura and Martínez-Millán [16]) (Baldwin <i>et al.</i> [21])
Landscape Shape Index (LSI)	Equals 0.25 times the sum of the entire forest boundary divided by the square root of the total landscape area	≥ 1	▲ ▼	(Shen <i>et al.</i> [23]; Wu [22])
Aggregation Index	Equals the number of like adjacencies involving the corresponding class (g_{ii}), divided by the maximum possible number of like adjacencies involving the corresponding class ($max-g_{ii}$), which is achieved when the class is maximally clumped into a single, compact patch (%)	$0 \leq AI \leq 100$	▼ ?	For the aggregation metrics (<i>sensu</i> McGarigal and Marks [44]) Baldwin <i>et al.</i> [21] reported a general decreasing function, while Wu <i>et al.</i> [20] reported unpredictable function
Clumpiness Index (CLUMPY)	Equals the proportional deviation of the proportion of like adjacencies (G_i) involving the corresponding class (P_i) from that expected under a spatially random distribution (%)	$1 \leq CLU \leq +1$		

The scaling behavior of the Number of Patches [21–23], the Total Edge [18,21,22] and the Shape Index [22,23] is well documented, whereas the relation between Mean Patch Size and the extent has been found to be unpredictable [22]. However, current information about the response of Edge Density, Aggregation, Clumpiness and Patch Density to changing scales is highly controversial (see Table 1 for details). Several studies have reported that Edge Density, Patch Density [16] Aggregation and Clumpiness [20] were insensitive or weakly sensitive to the spatial extent of the analysis, whereas several others have stated that they could show different types of scaling behaviors [21–23].

2.6. Map Simulations and Inference

In the real world, replications of a given landscape are often difficult to obtain because each single landscape shows a specific degree of land cover proportion and spatial autocorrelation [30]. To overcome the limited number of replications in natural landscapes, it is possible to rely upon simulations based on computer-generated models that serve to reproduce an expected pattern that shares statistical properties with an empirical pattern of interest [30]. Among the spatial models developed in ecology, Neutral Landscape Models (NLMs) can produce an expected pattern in the absence of specific landscape processes [45]. In this study, we used the Modified Random Cluster Method (MRC) implemented in the software SIMMAP 2.0 [32] to generate categorical (thematic) landscape spatial patterns in raster format (grid-based data). MRC is a stochastic simulation procedure that, through the variation of simulation parameters (the proportion of forest cover, fc , and the initial probability, p , which controls the degree of spatial autocorrelation), provides a wide range of simulated landscapes with intermediate levels of spatial dependence and in which the fragmentation and

abundance of land-cover classes can be systematically and independently controlled (see, for details, Saura and Martínez-Millán [32]).

First, for each year and extent, we simulated 15 maps (see Appendix A) that adequately characterized the mean values for the metrics considered [46]. We ran specific simulations for each extent and date, using their corresponding actual fc and p values as input. Because the initial probability p is not an explicit spatial pattern metric, we iteratively generated landscapes with different levels of p and then chose those simulated landscapes in which the level of autocorrelation, measured as Contagion H [47], was similar (based on a 99% confidence interval) to those of the observed real landscapes. Thus, by computing selected pattern metrics (Table 1) on both real and simulated landscapes, their empirical distributions (*sensu* Fortin *et al.* [29]) for each date and extent were derived. The empirical distributions, which are functions of the parameters used to generate the landscapes (fc and p), are often non-Gaussian but they provide the basis for determining confidence intervals [30]. Since pattern metrics are not statistics *per se*, that is their distributions are not derived analytically, to statistically compare them randomizations, resampling techniques or bootstrapping procedures are needed [48]. To compare real map pattern indices at different scales and time a bootstrap procedure (Bias-Corrected and accelerated bootstrap) was applied. By bootstrapping pattern metric values of each set of simulated MRC maps (three window sizes and two time periods) we derived the arithmetic mean and the 99% confidence intervals necessary to perform the significance testing [23,30,48]. If the confidence intervals (e.g., 99%) of a given spatial metric between different extents or time periods overlap, it can be stated that there are no significant differences between the compared landscapes. In order to better interpret the magnitude of the observed temporal processes, we measure the effect size of forest pattern change by computing the weighted average of the standardized difference (based on pooled variance measures) between mean metric values (for small medium and large windows) in 1954 and 2011 landscapes (that is, Hedges' g [49]). The effect size is positive when the metric value of the 2011 maps is greater than that of the 1954 ones and is negative when the metric value decrease in the recent time period. The magnitude of the effect size indicates which pattern metric has changed more than the others. We used a resampling procedure based on 10000 bootstrap samples (with replacement) to generate the mean effect size and 99% confidence intervals. All the analyses were performed in the R statistical computing program [50] by using the BootES package [51]. Then, for each metric, we built a multi-temporal scalogram by representing real map values (three map extents 128×128 , 256×256 and 512×512 pixels and two temporal periods 1954 and 2011) and the 99% confidence intervals (obtained by bootstrapping procedures on metric values of simulated MRC maps). Each multi-temporal scalogram reported the response curve of one pattern metric to changing extents on both the compared time periods (1954 and 2011).

3. Results

3.1. Pattern Metrics across Extents

The analysis of the multi-temporal scalograms underlined the existence of many significant differences between the compared extents of analysis and pinpointed specific behaviors of pattern metrics for each date (Figure 3; Table 2). Note that although the changes in the 2011 response curves were strongly linear, the

1954 curves appear to show a scale break (*sensu* Wu *et al.* [25]). For most of the metrics measured on the 1954 maps, an abrupt variation in the response curve at medium scale is evident.

Figure 3. Multi-temporal scalograms showing the effects of changing extent on forest pattern metrics in the two years analyzed (1954 and 2011). Lines connect real map metric values. Error bars denote the 99% confidence intervals for each pattern metric, obtained by applying bootstrap procedures (BCa) on simulated MRC maps. For methodological details, see Figure 2; for pattern metric acronyms, refer to Table 1. Type A, where the pattern metric curves for the compared data did not intersect and did not converge with each other; Type B, in which scalograms intersected each other and diverged; and Type C, with metric curves that converged but did not intersect.

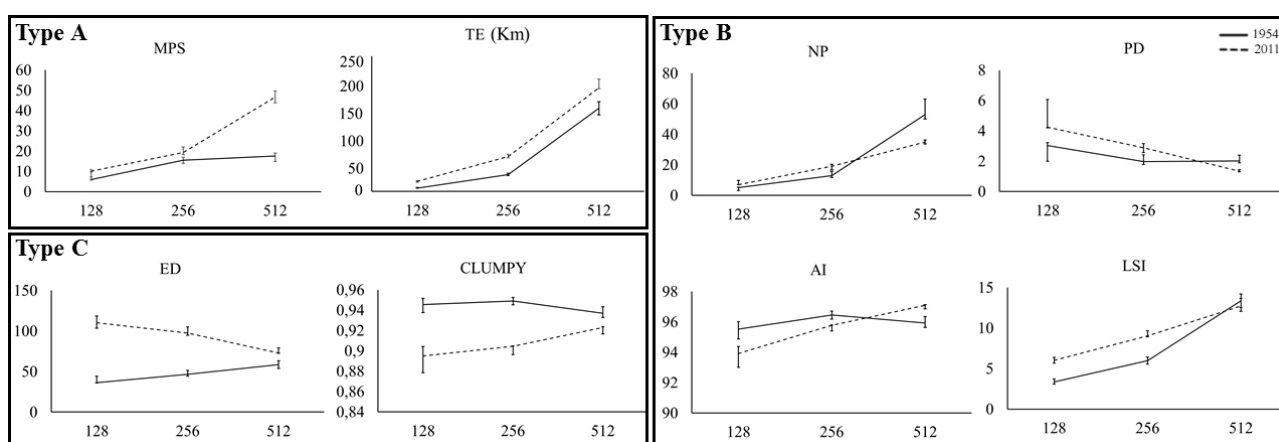


Table 2. Real values of pattern metrics obtained from three different map extents (128 × 128, 256 × 256 and 512 × 512 pixels) and two temporal periods (1954 and 2011) along with the 99% confidence intervals (CIs) obtained by applying bootstrapping procedures (BCa) on specific simulated MRC maps.

Landscape Metrics	1954				2011			
	Window Size	Real Value	Upper CI	Lower CI	Window Size	Real Value	Upper CI	Lower CI
Number of Patches (NP)	128 ^a	5 *	5.26	3.26	128 ^a	7 *	9.8	6.93
	256 ^b	13 *	15.73	11.73	256 ^b	19 *	20.42	16.79
	512 ^c	53 *	63.08	49.29	512 ^c	35 *	38.00	33.78
Patch Density (PD)	128 ^a	3.03 *	3.23	1.99	128 ^a	4.24 *	6.06	4.19
	256 ^a	1.98 *	2.42	1.79	256 ^b	2.89 *	3.15	2.56
	512 ^a	2.08 *	2.41	1.90	512 ^c	1.33 *	1.45	1.29
Mean Patch Size (MPS)	128 ^a	5.95 ns	9.71	5.61	128 ^a	10.07 ns	10.94	7.19
	256 ^b	15.53 *	16.92	13.88	256 ^b	19.33 *	22.05	18.27
	512 ^b	17.53 *	18.97	14.97	512 ^c	46.74 *	43.83	49.75
Total Edge (TE)	128 ^a	6000 *	7226	5871	128 ^a	18,200 *	19,414	16,953
	256 ^b	30,760 *	33,638	28,944	256 ^b	64,180 *	68,893	62,195
	512 ^c	153,680 *	165,624	141,188	512 ^c	191,730 *	207,541	190,012

Table 2.Cont.

Landscape Metrics	1954				2011			
	Window Size	Real Value	Upper CI	Lower CI	Window Size	Real Value	Upper CI	Lower CI
Edge Density (ED)	128 ^a	36.34 *	44.11	35.84	128 ^a	110.22 *	118.50	103.47
	256 ^b	46.75 *	51.33	44.17	256 ^a	97.55 *	105.12	94.902
	512 ^c	58.51 *	63.18	53.86	512 ^b	72.99 *	79.158	72.487
Landscape Shape Index (LSI)	128 ^a	3.37 *	3.73	3.07	128 ^a	6.04 *	6.39	5.64
	256 ^b	5.97 *	6.41	5.56	256 ^b	9.03 *	9.66	8.91
	512 ^c	13.37 ns	14.21	12.07	512 ^c	12.72 ns	13.63	12.55
Aggregation Index (AI)	128 ^a	95.53 *	96.01	95.53	128 ^a	93.93 *	94.39	93.01
	256 ^b	96.47 *	96.72	96.47	256 ^b	95.78 *	95.79	95.41
	512 ^{ab}	95.92 *	96.35	95.63	512 ^c	97.10 *	97.14	96.86
Clumpiness Index (CLUMPY)	128 ^{ab}	0.9455 *	0.9515	0.9376	128 ^a	0.8949 *	0.9043	0.8784
	256 ^a	0.9491 *	0.9526	0.9451	256 ^{ab}	0.9045 *	0.9049	0.8962
	512 ^b	0.9369 *	0.9434	0.9327	512 ^c	0.9230 *	0.9237	0.9166

^{a,b,c} Uppercase letters indicate significant differences among scales. * Asterisks indicate significant differences and ns, no significant differences, among time periods.

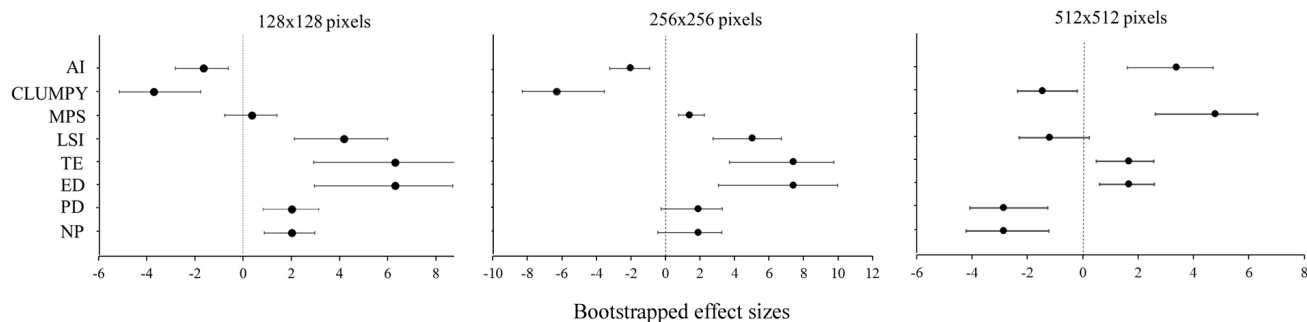
In the more recent landscape (2011), significant differences across all three compared extents were found for six of the eight metrics (except for ED and CLUMPY). In particular, ED significantly decreased between the small and the large extent as well as the medium and the large extent. The clumpiness index significantly increased from the smallest and the largest extents as well as between the medium and the large extents. The MPS, TE, NP, LSI and AI showed significant increases across the considered extents, whereas PD showed a significant decrease. The 1954 scalograms showed significant differences across all the extents for four of the analyzed parameters (TE, NP, SI and ED). All these metrics increased as the extent expanded. Mean Patch Size (MPS) significantly increased between small and medium extents as well as between small and large extents. The Aggregation Index (AI) significantly increased between the small and the medium extent, but no significant changes were evident between medium and large extents. Both patch density (PD) and Clumpiness Index (CLUMPY) tended to decrease across extents, but no significant changes were evident.

3.2. Pattern Metrics over Time

Comparing the maps for 1954 and 2011 (Figure 1), we found significant temporal changes in both the abundance and the spatial distribution of forests. Although the effect size varied between pattern metrics and scales (Figure 4). The magnitude of the effect sizes for each metric varies in correspondence of the different scales and tends to be higher on small and medium extents. For medium and small scales we found that TE and ED are the most sensitive metrics with an effect size substantially higher than that of the other metrics (Figure 4). For large extents, the most sensitive parameters are MPS and AI (Figure 4). Overall we found that all the significant changes on pattern metrics over time are relevant. The analysis of the multi-temporal scalograms (Figure 3) showed a strong influence of the spatial extent on forest pattern, and a specific response curve for the compared dates was also evident. The observed scaling relationships over time were schematically summarized in three main types of multi-temporal scalograms: Type A, where pattern metric curves for the compared data did not

intersect and did not converge with each other; Type B, in which scalograms intersected each other and diverged; and Type C, with metric curves that converged but did not intersect.

Figure 4. Effect size estimations. Mean values and 99% confidence intervals of the effect sizes estimation (10,000 bootstrap resampling) are reported. The vertical dashed lines represent an effect size of zero. For pattern metric acronyms, refer to Table 1.



Type A metrics tend to increase over time regardless of the extent of analysis. Total Edge (TE) and Mean Patch Size (MPS), increased between 1954 and 2011 across all extents, and belonged to this group. However, note that the MPS values at the smallest extent were not significantly distant from each other (99% confidence interval overlap between them). The scalograms of Type B intersected each other; furthermore, depending on the chosen extent of analysis, opposite temporal changes in the spatial metrics emerged. Interestingly, most of the metrics belong to this Type B group: Number of Patches (NP), Patch Density (PD), Aggregation Index (AI) and Shape Index (SI). For example, the curves describing Number of Patches (NP) and Patch Density (PD) relative to the years 2011 and 1954 intersected each other after the medium extent. Thus, the analysis of forest pattern at medium and small extents showed significant increments in the number and density of forest patches. In contrast, at the largest extent, a significant decrease in the number and density of patches between 1954 and 2011 was found. SI showed similar behavior, but the decline in the 2011 map was not significant at the largest extent. For the Aggregation Index at smaller extents, the 1954 curve was higher than the 2011 curve, whereas the 1954 curve dropped below the 2011 curve at the largest extent. Specifically, the AI values decreased significantly between 1954 and 2011 at smaller extents but significantly increased at the largest extent. In the Type C scalograms, the 1954 and 2011 curves tended to converge but maintained significant distances at all the investigated spatial extents and did not intersect. Type C curves included ED and CLUMPY. In particular, CLUMPY significantly increased over time as ED significantly decreased.

4. Discussion

The observed increase in forest cover over the past 60 years, along with the significant changes in forest spatial pattern and the effect sizes analysis, suggest that the analyzed area has undergone an intense process of natural recolonization that began after World War II and that is still in progress. The phenomenon that we observed could be considered reforestation (*sensu* Sitzia *et al.* [24]), *i.e.*, the natural reestablishment of a forested landscape on disused agricultural lands following farm abandonment in regions where the potential natural vegetation (*sensu* Zerbe [38]) is a forest.

The statistical comparison of pattern metrics at different window sizes over time allows the recognition of the characteristic extent, highlighted by the scale breaks, at which specific patterns of the ongoing process of reforestation are more evident. For the compared dates, significant variations in many metric values were found to correspond with different map extents. The multi-temporal scalograms of type A summarize the behavior of metrics (MPS and TE) that describe similar temporal changes in forest pattern regardless of the extent of analysis. Even if such parameters can be sensitive to the map boundary effect [21,52], they unambiguously depict the ongoing landscape process of forest regrowth in our case. The increase in patch size and edge length over time suggests that forest regrowth has occurred evenly over the entire landscape. At small extents, the absence of significant differences in patch size in association with an increase in the total edge length depicts a landscape with many small patches that, most likely, are the new nuclei of young forests [34]. In the multi-temporal scalograms of type B, the curves intersect each other due to the presence of a scale break at the medium extent for the 1954 scalograms. The curves of type B include parameters such as NP, PD, AI and LSI that describe opposite temporal trends in the pattern of forests depending on the extent of analysis. Such findings serve as a warning to researchers and planners. If these parameters are used over time, it is strongly recommended to analyze them at multiple scales to avoid misleading or partial conclusions. In our case, the parameters of type B describe different aspects of the forest regrowth process. At small and medium extents, the general increase in the number of patches and their spatial density over time describe the ongoing process of natural recolonization. Indeed, the establishment of several new forest nuclei is characteristic of the natural colonization of abandoned lands in Mediterranean ecosystems [34]. In contrast, the observed decrease at larger extents in NP, PD and SI describes the expansion and the coalescence of several secondary forest patches into larger ones [31,34,35,53]. Similar behavior is also evident for the AI index. At smaller extents, decreasing values of AI pinpoint the typical disaggregated pattern that characterizes the initial stages of natural forest regrowth [34]. In contrast, at the larger extent, the significant decline in forest pattern aggregation over time highlights the process of coalescence of forest patches and the consequent increase in forest connectivity. In the scalograms of type C, the 1954 and 2011 curves are significantly distant but tend to converge at the largest extent. The growth of ED over time and the reduction of CLUMPY values clearly indicate a more dispersed distribution of present-day forests relative to past forests. The convergence of ED values in association with a significant increase in forest cover—from 35% (1954) to 62% (2011)—is most likely related to the parabolic distribution of the index as a function of forest cover [54,55]. In particular, ED values increase as forest cover expands and peak when the proportion of forest reaches 50% of the landscape extent. For this reason, markedly different landscapes exhibit very similar ED values. On the other hand, the significant decrease in CLUMPY values over time and across all the extents reveals an increase in forest dispersion. Most likely, the process of natural forest recolonization in abandoned lands occurs in a stochastic manner [34].

Overall, the observed differences in the scaling behaviors over the compared time periods are most likely related to the various ways in which humans exploited landscape resources in the compared years. In 1954, for example, land-based economic activities (such as grazing and agriculture) had forced forests into areas in which productivity was low [36] promoting the development of an anisotropic pattern. Instead, the more recent process of natural reforestation has been driving the entire landscape toward a more natural and homogeneous pattern [35]. Forest regrowth on abandoned lands

occurs in a stochastic manner [34], with patches that expand isotropically (in all directions) and tend to be uniformly distributed over the entire landscape (statistically stationary). In such situations, pattern metrics manifest predictable and simple scaling relations. Note that the obtained results are strongly dependent on the specific type of landscape, which, in our case, is characterized by a homogeneous underlying environmental structure [36] (geology, morphology, climate, soil). Indeed, we are observing the natural reestablishment of a forested landscape in regions where the potential natural vegetation (*sensu* Zerbe [38]) is a temperate forest. Different results should emerge in landscapes with high environmental heterogeneity, in which, by tuning the extent of analysis, specific scalograms and metric behaviors could emerge.

Many of the understandings and conclusions obtained in this study have been facilitated by the proposed statistical framework. The chosen modeling procedure, which incorporates the temporal variation in landscape composition (forest cover) and configuration (spatial autocorrelation) occurring in real landscapes [16,19] offers useful insights to address the influence of spatial extent on pattern variation over time. In particular, the utilization of the observed proportions of forest (fc) and the values of spatial autocorrelation (H) as input parameters for the stochastic simulation procedures allows an adequate description of the process of forest regrowth and, at the same time, has yielded a robust statistical and defensible framework. In particular, the application of the MRC algorithm allows the following approaches: (i) modeling a plausible set of maps, with different levels of forest proportion and patchiness, that adequately describes the spatial pattern of forests through time and across scales; (ii) generating a set of landscape replications that recognizes the most relevant real landscape information; (iii) defining the landscape expectations, allowing the statistical comparison of patterns through time and across scales; and (iv) avoiding the effects of the window direction of analysis.

5. Conclusions

Although many authors have stressed the importance and limitations of employing pattern metrics for comparing landscapes [56], the use of these metrics for characterizing and monitoring forest distribution over time continues to be highly popular [57,58]. We proposed and tested a procedure to detect significant changes in forest spatial patterns and relevant scales. This approach enriches the set of the existing methods for multi-scale/multi-temporal landscape studies by including the statistical analysis of the observed differences. As a demonstration, we analyzed the change in the spatial pattern of temperate forests in a Mediterranean hilly landscape over the last 60 years across different extents.

Our results highlight that if landscape pattern is analyzed at a single extent that does not match the scale at which a given phenomenon occurs (e.g., reforestation), the results are incomplete and obscure the effective landscape variation over time. For example, we found that different patterns of the ongoing process of natural reforestation emerged (e.g., nucleation and coalescence of the existing patches in a unique bigger one) at different spatial extents.

The proposed multi-temporal analysis, which incorporates the effects of scale on pattern metrics and the statistical significance of the differences in metric values, have helped to relate the changes in pattern parameters to landscape processes. It overcame and minimized the potential bias introduced in traditional studies that simply resort to the comparison of pattern metrics at a single extent, ignoring information about the distribution and variability of the pattern metrics.

Even if the obtained results are strongly dependent on both the specific type of landscape and the chosen spatio-temporal scales, analogous methods could be used for the study of pattern changes and statistical expectations across a large ensemble of landscapes. In any case, consistent scale breaks could be expected in strongly human-shaped landscapes, where anthropogenic driving forces lead to the juxtaposition of different ecosystems (natural, semi-natural and artificial), whereas linear scaling relations should emerge in more natural landscapes.

From a practical point of view, the obtained results offer scientifically sound bases for orienting decisions in various fields, such as forest management and monitoring. For example, the correct choice of the spatial extent might help to better define conservation measures oriented to increase landscape connectivity values [59]. Furthermore, the proposed approach could allow for the examination of the long-term effects of the extent of the protected area on forest distribution and other conservation features, which is essential for assessing their effectiveness [60,61].

We believe that similar procedures, designed to perform statistically robust multi-temporal and multi-scale analyses, could become a standard method for the comparison of categorical maps, especially if the investigated landscapes, are samples extracted from areas of fixed size and shape [6]. Such procedures are particularly necessary in the consideration of change detection and when uncertainties about the scale and pattern metric values exist and could provide relevant indications regarding the changes in landscape structure over time and all the ecological and cultural consequences linked to this issue.

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Author Contributions

Conceived and designed the experiments, analyzed the data and wrote the manuscript: Ludovico Frate, Maria Laura Carranza. Produced data and reviewed the drafts: Michele Minotti, Carmen Giancola, Paolo Di Martino. Discussed main topics and reviewed the text: Santiago Saura.

Conflicts of Interest

The authors declare no conflict of interest.

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SEZIONE II.

Measuring Forest Fragmentation Over Time: Applications in Subtropical Forest Landscapes

CAPITOLO 3

CARRANZA M.L., FRATE L., ACOSTA A.T.R., HOYOS L., RICOTTA C., CABIDO M. 2014. Measuring forest fragmentation using multitemporal remotely sensed data: three decades of change in the dry Chaco. *European Journal of Remote Sensing* 47: 793-804. doi: 10.5721/EuJRS20144745



Measuring forest fragmentation using multitemporal remotely sensed data: three decades of change in the dry Chaco

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Abstract

We introduce an approach based on remotely sensed data to summarize forest fragmentation over time, which specifically accounts for the interdependencies between landscape composition and configuration changes. The proposed method consists of five steps: i) multitemporal landscape sampling, ii) calculation of selected landscape pattern indices, iii) statistical comparison, iv) construction of sampled-based relationship spaces, and v) trajectory analysis. To show how the proposed method works in practice we examined the multitemporal fragmentation of the Arid Chaco forest in central Argentina during the period 1979-2010 using forest maps derived from Landsat images. As shown by our results, the approach provides a consistent framework for the interpretation of landscape structural changes over time.

Keywords: Chaco dry forests, forest loss, Landsat, landscape metrics, sample-based analysis, trajectory analysis

Introduction

Forest ecosystems have played a major role in human history and forest fragmentation has accompanied population growth and development throughout the world for thousands of years [FAO, 2012]. Forest fragmentation is a landscape-level process in which a large intact forest area is progressively divided into smaller, geometrically altered and isolated patches [Forman and Godron, 1986; Fahrig, 2003]. Anthropogenic fragmentation of natural forests constitutes one of the most severe causes of biodiversity loss [Foley et al., 2005; Wade

et al., 2003] and of the impairment of forest ecosystem services [Marchetti et al., 2012; Gamefeldt et al., 2013]. The remarkable speed of forest fragmentation all over the world urges to find standard screening procedures able to stress the benefits and drawbacks of different management scenarios [Gamefeldt et al., 2013]. Scientifically sound instruments able to describe and monitor forest fragmentation are crucial for determining conservation priorities aimed at guaranteeing forest biodiversity and ecosystem services over time [Gomez-Sanz et al., 2008].

Remotely sensed imagery is the most successful tool for forest cover monitoring, as it offers a cost-effective option for frequent observations of vast areas [Potapov et al., 2012]. Moreover, remote sensing is particularly effective for producing forest cover maps (for a throughout review see Achard and Hansen [2012]). Forest maps provide explicit information on forest distribution [Stehman, 2012], which is the first step of fragmentation analysis.

Forest fragmentation consists of two interdependent components: forest loss and changes in spatial configuration [Neel et al., 2004; Long et al., 2010]. Accordingly, a proper interpretation of forest fragmentation needs to consider the interdependencies among these aspects [Neel et al., 2004]. Indeed, while the reduction of forest cover is usually accompanied by a change in the spatial configuration of the remaining forest fragments, a large body of class-level configuration metrics (hereafter referred to as LPIs) [McGarigal and Marks, 1995] is highly correlated with habitat abundance [Neel et al., 2004; Cushman and McGarigal, 2007]. In order to investigate the nonlinear and non-monotonic relationship between habitat cover and LPIs in deeper detail, Long et al. [2010] proposed the use of specific bidimensional ‘relationship spaces’ in which the variation of LPIs, such as forest fragmentation, can be plotted against different levels of class proportions (i.e. forest cover). In their seminal paper, Long et al. [2010] used neutral simulation models to reproduce the relationship between pattern metrics and habitat proportions. However, due to their intrinsically random nature, neutral simulation models are usually unable to reproduce the fragmentation patterns of highly disturbed landscapes where the anthropogenic forces give rise to severely constrained spatial distributions [Li et al., 2004]. In this context, the use of sample data obtained from remotely sensed imagery may represent a valuable alternative. For instance, by projecting the LPI values measured on sample data in a bidimensional relationship space it is possible to depict the observed real-world association between habitat cover and configuration metrics of a given landscape at a given point in time.

The aim of this paper is thus to propose an approach based on remotely sensed data to summarize forest fragmentation over time, which specifically accounts for the interdependencies between landscape composition and configuration changes. In particular, we suggest to quantify changes in forest loss and spatial configuration using random sampling of multi-temporal maps followed by a bootstrapping significance test. As an application for demonstration, a multi-temporal analysis of forest fragmentation in the Argentinean dry Chaco is performed.

Analyzing forest fragmentation over time

The proposed approach for assessing forest fragmentation over time can be described in five steps: i) multitemporal landscape sampling, ii) LPIs selection and calculation, iii) statistical comparison, iv) construction of the sampled-based relationship spaces and v) multitemporal trajectory analysis of the LPIs estimators.

(i) Multitemporal landscape sampling

The application of sampling methods for analyzing landscape-scale forest configuration may yield significant cost savings and more accurate results when the analyzed area is extensive [Ramezani et al., 2013]. The sample-based method consists in sampling the study area with the objective of achieving approximately what would happen if the classical wall-to-wall data were analyzed [Stehman, 2012]. In its very essence, for fragmentation analysis, we propose to extract randomly a finite population of n sampling units from a grid of N non-overlapping cells (e.g. $1 \text{ km} \times 1 \text{ km}$ or $10 \text{ km} \times 10 \text{ km}$ units) in which the study area is partitioned. For a review of the strengths and weaknesses of coarse-scale sample-based methods for forest monitoring see Stehman [2012].

(ii) LPI selection and calculation

Partitioning a region into spatial units and then selecting a subset of these units introduces artificial patch edge and patch truncation effects that may lead to biased sample-based estimators of landscape pattern metrics [Hassett et al., 2012]. Only a limited set of LPIs are adequate for sample-based analysis of landscape structure [Hassett et al., 2012; Ramezani et al., 2013]. In particular, the sample-based analysis of the percent cover of a given landscape class and of its edge density offers unbiased estimators of the entire landscape [Stehman et al., 2003], while the bias of the estimators of mean patch size and patch density is very small or negligible [Hassett et al., 2012]. All those parameters, which are also among the most used indicators for fragmentation analysis [Townsend et al., 2009; Moreno-Sanchez et al., 2012; Frate et al., 2014], can be easily calculated for each sampling unit using off-the-shelf software, such as FRAGSTATS [McGarigal and Marks, 1995].

(iii) LPIs estimation and statistical comparison

Once the fragmentation LPIs of each sampling unit have been calculated, a variety of estimators can be used to assess the parameter of interest. For probability sampling designs and design-based inference, a general unbiased estimator of a population total is the Horvitz-Thompson estimator [Overton and Stehman, 1995]. Imagine a landscape that is entirely tessellated into N non-overlapping units, and let θ denote the value of a landscape pattern metric computed from complete wall-to-wall land cover data for the region of interest and θ^* the mean value of the metric for the universe N . The Horvitz-Thompson estimator allows to construct an unbiased estimator $\hat{\theta}^*$ for any probability sampling design of n units out of N . An important advantage of the Horvitz-Thompson estimator is that for the special cases of the basic sampling designs typically used in practice (e.g. simple random sampling, systematic sampling, or stratified random sampling), the Horvitz-Thompson estimator reduces to simplified formulas. For instance, for a simple random sampling design the estimator reduces to the arithmetic mean of the n sampling units:

$$\hat{\theta}^* = 1/n \sum_1^n z_n \quad [1]$$

where Z_n is the value of the metric computed for the n -th sampling unit. For a detailed description of the Horvitz-Thompson estimator for probability sampling designs and

design-based inference see Stehman [2012]. As a next step, to test for differences among landscape metrics of fragmentation, bootstrap procedures [Manly, 2006; Fortin et al., 2012] can be used.

(iv) Construction of the sample-based relationship space

To visualize the nonlinear relationship between forest cover and landscape metrics, the index values of all sampled spatial units can be projected against the corresponding forest cover proportions in order to build index-specific relationship spaces [sensu Long et al., 2010]. Such relationship spaces not only provide a sound frame for the analysis of fragmentation over time but are also useful for describing the spatial consequences of forest loss.

(v) Trajectory analysis

Landscape trajectory analysis, introduced by Cushman and McGarigal [2007], consists in describing the position of a given landscape over two or more observation periods in the corresponding multidimensional LPI space. Here, we propose to perform trajectory analysis in the index-specific bidimensional relationship space of Long et al. [2010] to provide an intuitive and interpretable description of forest fragmentation over time. Once the LPIs estimators of a number of observation periods are plotted in sample-based relationship space, temporal trajectories can be drawn connecting the corresponding point as time-ordered series. When, temporal changes are moderate, the fragmentation estimators are located very close in the relationship space. To the contrary, in highly dynamic landscapes, the position of the fragmentation estimators in the relationship space tends to diverge over time.

Worked example

Study area

A test site of roughly 2713 km² of the Gran Chaco dry forest located in central Argentina was selected for the analysis (Fig. 1). The climate is warm temperate to subtropical, with a mean annual temperature ranging from 16°C to 19°C and mean annual rainfall ranging from 400 to 800 mm [Zak et al., 2008]. The Gran Chaco, is one of largest seasonally dry subtropical forests in the world (ca. 1200000 km²) and comprises wide areas in Argentina, Paraguay and Bolivia where the transition between the tropics and the temperate belt does not occur in the form of a desert but as semi-arid forests and woodlands [Morello and Adamoli, 1974; Zak et al., 2008]. The study area was formerly dominated by *Aspidosperma quebrachoblanco* and *Schinopsis marginata* subtropical seasonally dry forests [Sayago, 1969; Zak and Cabido, 2002]. Despite many outstanding features in terms of biodiversity values [Molina et al., 1999; Cagnolo et al., 2006; Torrella et al., 2013] and ecosystem services [Conti and Diaz, 2013; Cáceres, 2014], which make these complex ecosystems worthy of protection, the Gran Chaco, is one of the main deforestation areas of Latin America [Grau and Aide, 2008; Hansen et al., 2012]. During the last three decades the generalized expansion of agriculture [Zak et al., 2008; Hoyos et al., 2013], driven by global trends in technology and soybean markets [Grau et al., 2005], but also by global changes in the precipitation regimes [Hoyos et al., 2013], have promoted a sharp drop of the Gran Chaco natural forests.

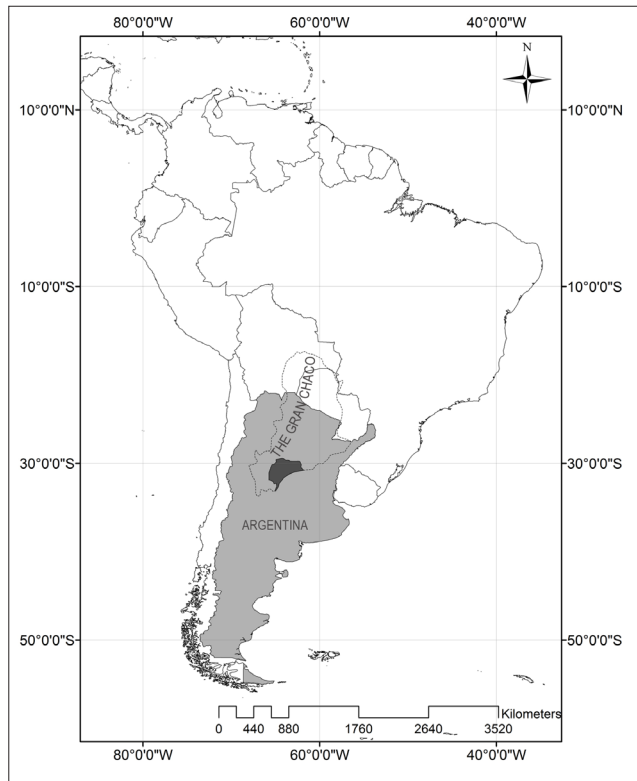


Figure 1 - Location of the study area (in dark grey). The Gran Chaco biogeographical region (black-dashed line) and the administrative limits of Argentina (light grey) area also shown.

Methods

Based on Landsat satellite images for the years 1979, 1999 and 2010 and extensive field work three large-scale land cover maps for the study area were produced. To identify the land-cover units, three Landsat MSS scenes from February 1979, three Landsat TM scenes from November 1999, and three Landsat TM scenes from March 2010 were used. All Landsat images were acquired during the vegetation growing season of the southern Hemisphere. The classification of Landsat MSS and TM images resulted in reliable land-cover maps (overall accuracy 80%) composed of five vegetation classes: closed forest, open forest, shrublands, halophytic vegetation and cultural vegetation (croplands and urban areas). For a detailed description of the classification procedure, see Hoyos et al. [2013]. The subsequent fragmentation analysis was performed solely on the closed forests class. These forests correspond to lowland seasonally dry forests, with *Aspidosperma quebrachoblanco* and *Schinopsis lorentzii* as dominant trees and a canopy cover of at least 50% [Cabido et al., 1992; Zak and Cabido, 2002]. A set of non-overlapping square grid units was randomly sampled without replacement from the tessellated study area (roughly amounting to 10% of the total extent of the analyzed land cover class). The analysis was performed at two grid dimensions [see Long et al., 2010], comparable with those commonly used for

mid-scale and coarse-scale regional forest monitoring [Wulder et al., 2008]: 1 km² (1692 sampling units) and 10 km² (191 sampling units). For each sampling unit, a set of four indices of landscape fragmentation was computed with FRAGSTATS [McGarigal and Marks, 1995]. These indices include: percent of forest cover (% Forest), edge density (ED; m/ha), mean patch size (MPS; ha) and patch density (PD; number of patches/ha). The detailed formulas of the LPIs used in this paper can be found in McGarigal and Marks [1995]. For each grid size three index-specific relationship spaces were built by projecting the values of ED, PD and MPS computed for each sampling unit against the corresponding forest cover values. The Horvitz-Thompson estimators of all LPIs were then calculated as the arithmetic mean of the *n* cells sampled at each date. The LPI estimators were finally plotted in the corresponding sample-based relationship spaces to describe the temporal trajectory of each index. Temporal differences between the LPI estimators were statistically tested with a bias-corrected and accelerated bootstrap procedure [Manly, 2006; Fortin et al., 2012].

Results

The sample-based analysis of the Arid Chaco forest over time underlines a consistent process of fragmentation. The LPI temporal trajectories in relationship space for both grid dimensions are shown in Figure 2 and Table 1. During the last 30 years, a significant decline of forest cover and a consistent change in forest spatial configuration can be observed. As shown in Table 1, the results obtained for the 1 km² grid are very similar in sign and strength to the results of the 10 km² grid. Therefore, for simplicity, in this section we report only the LPI values associated to the smaller grid size.

Table 1 - Horvitz-Thompson estimators of the sample-based LPIs used in this study for the years 1979, 1999 and 2010 and for two grid sizes: 1 km² and 10 km². The 99% confidence intervals obtained by the bias-corrected and accelerated bootstrap procedure are also shown. Different lowercase letters on the right side of the index mean values show significant pairwise differences between the observation periods at *p* = 0.01. PLAND: percentage of forest cover (%), MPS: mean patch size (ha), ED: edge forest density (m/ha), PD: patch density (number of patches/ha).

		1979			1999			2010		
		Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
1 km ²	PLAND	31.20 ^a	30.03	32.39	23.29 ^b	21.90	24.66	4.19 ^c	3.77	4.63
	MPS	13.98 ^a	12.81	15.14	12.72 ^a	11.53	14.01	0.79 ^b	0.61	0.98
	ED	60.75 ^a	59.21	62.25	39.06 ^b	37.25	40.89	15.63 ^c	14.33	16.89
	PD	6.00 ^a	5.82	6.19	3.76 ^b	3.57	3.94	3.22 ^c	3.00	3.45
		1979			1999			2010		
10 km ²	PLAND	33.55 ^a	30.44	36.74	23.82 ^b	20.36	27.44	3.48 ^c	2.68	4.30
	MPS	62.22 ^a	38.42	87.91	32.77 ^a	16.93	50.35	0.70 ^b	0.58	0.83
	ED	64.74 ^a	61.09	68.60	41.79 ^b	37.02	46.68	14.29 ^c	11.48	17.11
	PD	4.19 ^a	3.83	4.53	2.64 ^b	2.33	2.96	2.77 ^b	2.30	3.26

Forest cover consistently declined from roughly 31% of the 1979 landscape to ~ 4% in 2010. Forest loss occurred at different rates during the analyzed time period. In the first

two decades (1979-1999) forest cover decreased from ~31% to ~23%, while in the last ten years (1999-2010), a much larger forest loss (from ~23% to ~4%) was observed. At the same time, fragmentation metrics are characterized by index-specific behaviors in relation to forest cover (Fig. 2). For instance, as shown by the relationship space of MPS vs. % Forest (Fig. 2), mean patch size tends to be very low for forest cover values below 50%; higher MPS values are observed only for forest cover values > 50%.

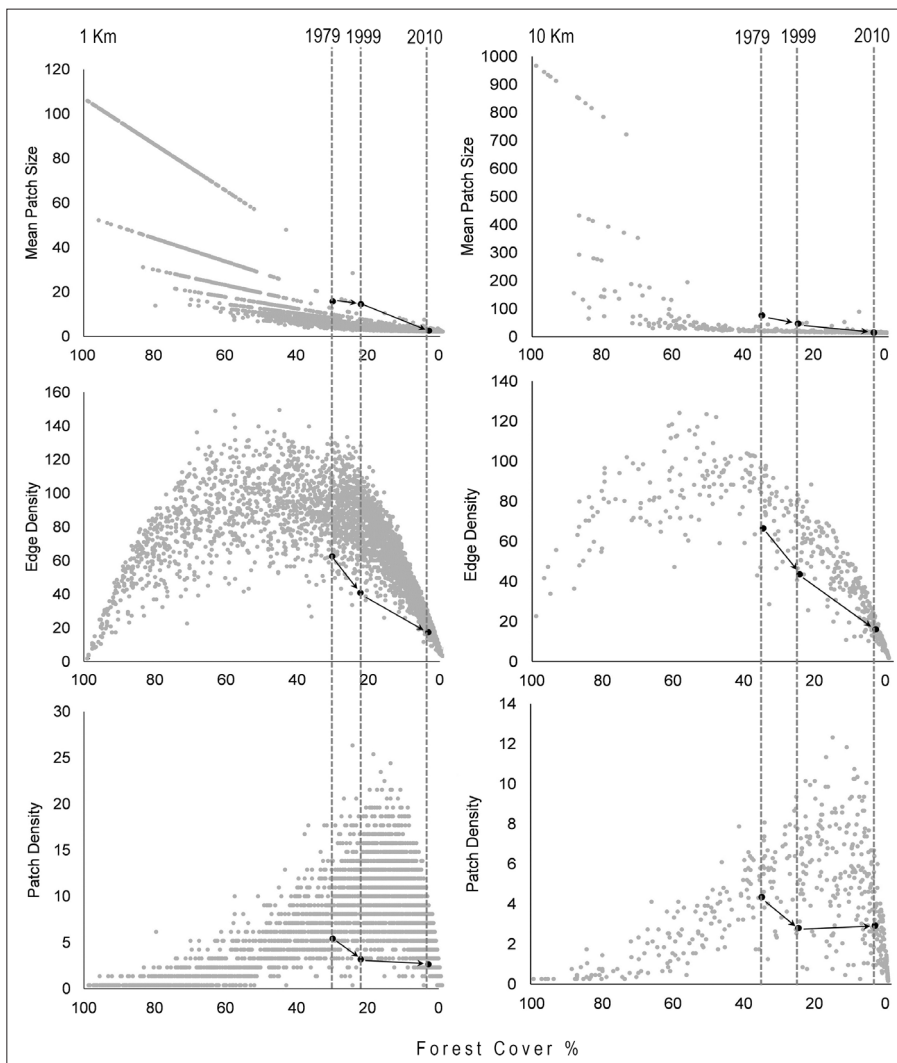


Figure 2 - Trajectory analysis of the Chaco dry forests trough the years 1979, 1999 and 2010 in the relationship spaces given by the LPI values vs. forest cover. MPS: mean patch size (ha), ED: edge forest density (m/ha), PD: patch density (number of patches/ha). Grey circles represent the LPI values of all square grids sampled in the tree years of observation. Black circles are the Horvitz-Thompson estimators of each index for each year of observation. Solid lines indicate the trajectories between successive dates.

The multitemporal analysis of the mean MPS values highlights significant changes in the 1999-2010 period (MPS ranges from 12.72 in 1999 to 0.79 in 2010; $p = 0.01$), whereas no significant changes were observed in the 1979-1999 period. Therefore, although MPS is among the most commonly used parameters for a wide range of landscape-level applications [e.g. Batistella et al., 2003; Fahrig, 2003; Frohn and Hao, 2006], our results are consistent with previous studies of Neel et al. [2004], which considered MPS as a parameter of little use for highlighting structural differences among landscapes with comparable cover values, especially at low cover levels.

As shown in Figure 2, Edge density is characterized by a parabolic relationship with forest cover. ED is low at very high and very low forest cover values and peaks at intermediate values of forests cover. The ED estimators for the years 1979, 1999 and 2010 show a significant, decline over time, ranging from 60.75 in 1979 to 15.63 in 2010. Overall, ED is a widely used parameter for fragmentation analysis, especially for the ecological implications of 'edge effects' [Saura and Martinez-Millan, 2001]. In this view, our results highlight the effectiveness of this index for comparing structural changes in highly fragmented landscapes of low forest cover, such as the Arid Chaco.

Finally, patch density also shows an (asymmetric) parabolic distribution in the corresponding relationship space with peaking values around 20% of forests cover (Fig. 2). The index estimators for the period 1979-1999 show a significant reduction in the number of forest patches (from an average of 6 patches/ha in 1979 to 3.76 patches/ha in 1999; $p = 0.01$). After this date, mean patch density remained more or less constant, although forest cover decreased from ~23% to ~4%.

Discussions

In this paper, we outlined the recent history of forest cover change in the study area of the Gran Chaco. The results of the fragmentation analysis clearly depict a devastating situation of these dry forests and its progressive reduction to few small fragments during the last decades. The forest landscape changed significantly between 1979 and 2010 and deforestation processes are probably still active. A thorough discussion of the ecological consequences of the observed forest loss is beyond the scope of this paper, but see Grau et al. [2005], Zak et al. [2008], Caldas et al. [2013], Hoyos et al. [2013] for details.

From a more general viewpoint, our findings underline the potential role of sample-based relationship spaces for fragmentation analysis. The proposed approach effectively describes the relationship between forest loss and landscape structural changes and offers a sound framework for a correct interpretation of forest fragmentation processes, while the high number of replicates enables the calculation of reliable confidence intervals and hence the statistical comparison between multitemporal maps.

The decreasing trend of mean patch size (MPS) as a function of forest loss, renders MPS an effective metric for describing changes in fragmentation pressure. To the contrary, the parabolic relationship of patch density (PD) and edge density (ED) with forest cover, limits their diagnostic potential to landscapes where forest cover are comprised within certain abundance ranges. Overall, while there are no perfect metrics for fragmentation analysis, many fragmentation indices might be useful under certain conditions and for answering specific biological questions. In this view, our findings suggest to carefully investigate the relationships between configuration metrics and forest cover, paying particular attention to

their nonlinear behavior.

From an applied perspective, the construction of sample-based relationship spaces provides valuable information for land management and fragmentation prevention issues. For instance, efficient conservation programs of forest biodiversity in changing landscapes could benefit from a multitemporal landscape trajectory analysis. Being based on remotely sensed data, the proposed procedure has a strong potential for performing continuous monitoring of landscape fragmentation in an efficient and affordable manner. For instance Earth observation satellites, such as Landsat, SPOT or MODIS, already support many landscape ecological studies from local to global scales at moderate cost [Townsend et al., 2009; Achard and Hansen, 2012; Fichera et al., 2012; Gargano et al., 2012; Schucknecht et al., 2013; Almeida et al., 2014]. Bearing this in mind, we hope our approach will be useful for providing early-warning signals of potential threats to forest integrity and sustainability at increasingly larger scales.

Acknowledgements

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CAPITOLO 4

CARRANZA M.L., HOYOS L., FRATE L., ACOSTA A.T.R., CABIDO M.

Measuring forest fragmentation using multitemporal forest cover maps: forest loss and spatial pattern analysis in the Gran Chaco, Central Argentina. *Landscape & Urban Planning, Under Review.*

Manuscript Number:

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Abstract: Forest fragmentation is a landscape-level process that consists of two interdependent components: forest loss and spatial pattern changes to which species respond differently. Efficient programs to conserve native biodiversity require a sound understanding of the relation between forest cover and the spatial pattern of forest fragments, but these issues remain almost unknown for subtropical ecosystems. We examine the forest fragmentation of the Gran Chaco in central Argentina over the last 30 years. In particular, we quantify forest loss and spatial pattern changes using random sampling techniques on multi-temporal forest cover maps (1979, 1999 and 2010). We analyzed forest fragmentation according to the following steps: (i) selection of fragmentation pattern indices (PIs), (ii) sampling on forest cover maps and PIs calculation, (iii) statistical comparison by bootstrapping, and (iv) trajectory analysis. During the last three decades, forest cover declined dramatically (~90%) and the selected pattern metrics (MPS, PD, ED) vary significantly ($p= 0.05$). The results depict a devastating situation of dry Chaco forests with a progressive reduction to few small fragments during the last decades. Distinguishing habitat spatial pattern changes from forest loss supports the identification of specific conservation actions necessary to mitigate the effects of fragmentation in these ecosystems

Measuring forest fragmentation using multitemporal forest cover maps: forest loss and spatial pattern analysis in the Gran Chaco, Central Argentina

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Highlights

- We quantify forest loss and spatial pattern changes by sampling cover maps
- Pattern metrics shows index-specific relationship with forest cover
- Significant forest loss occurs along with significant changes in pattern metrics
- Useful information for land management and fragmentation-prevention issues

1 **Introduction**

2 Forest ecosystems have played a major role in human history, and forest fragmentation
3 has accompanied population growth and development throughout the world for thousands of
4 years (Food and Agricultural Organization of the United Nations, 2012). The extent of
5 fragmentation, which has affected many natural forests worldwide, constitutes one of the most
6 serious causes of biodiversity loss, which in turn greatly influences ecosystem structure and
7 function. Recent studies have indicated that fragmentation has several negative (Trombulak et al.,
8 2004) and long-lasting (Flaspohler et al., 2010; Turner, 1996) environmental and ecological
9 consequences: it affects ecosystem functions, such as hydrological cycles and soil dynamics
10 (Rudel et al., 2005), climate regulation (Houghton et al., 2000; Nabuurs, Schelhaas, Mohren, &
11 Field, 2003) and biodiversity (see Fahrig, 2003 for a review).

12 According to the patch-corridor-matrix model (Forman, 1995), forest fragmentation can
13 be seen as a landscape-level process in which a large, intact area of a single forest type is
14 progressively sub-divided into smaller, geometrically altered and isolated patches (Fahrig, 2003;
15 Forman & Godron, 1986, McGarigal, Cushman, & Regan, 2005). Forest fragmentation consists
16 of two components: forest loss and changes in the spatial pattern (i.e. pattern metrics) (Fahrig,
17 2003; Long, Nelson, & Wulder, 2010; Neel, McGarigal, & Cushman, 2004). Forest loss and
18 spatial pattern changes are the two most important factors in the current species extinction event
19 at global scale (Fahrig, 1997; McGarigal, Cushman, & Regan, 2005). In response, there is a
20 growing mandate among natural resource managers to evaluate the impacts of proposed
21 management actions on habitat fragmentation (Long, Nelson, & Wulder, 2010; Wang, Blanchet,
22 & Koper, 2014). Thus, new guidelines to help managers to understand the many complex issues
23 involved in the assessment of habitat fragmentation are urgently needed. For instance, if in a

24 given landscape forest loss results in a constant number of smaller patches, then fragmentation
25 effects on biodiversity are due to forest loss alone. Only when the number of patches increases by
26 the breaking apart of forests do we find that both forest loss and spatial pattern (decreasing size
27 and increasing isolation of forest patches) are involved. On the other hand when forest amount is
28 constant over time, changes in spatial pattern generally has either no effect or a negative effect on
29 forest species survival (Fahrig, 1997; Gavish, Ziv, & Rosenzweig, 2012)

30 The Gran Chaco, which is among the largest seasonally dry subtropical forests in the
31 world, (ca. 1 200 000 km²), occurs in Argentina, Paraguay and Bolivia (Bucher 1982; Zak,
32 Cabido, Cáceres, & Diaz, 2008). It comprises one of the few areas worldwide where the
33 transition between the tropics and the temperate belt does not occur in the form of a desert but as
34 semi-arid forests and woodlands (Morello & Adamoli, 1974; Prado, 1993). These subtropical
35 seasonally dry forests are characterized by a specific vegetation and fauna that determines
36 consistent biodiversity values (Molina, Valladares, Gardner, & Cabido, 1999; Cagnolo, Cabido,
37 & Valladares, 2006; Torrella, Ginzburg, Adámoli, & Galetto, 2013). Moreover, these forests
38 provide numerous ecosystem services (Conti & Diaz, 2013) that are necessary for the subsistence
39 of local communities and the regional economy (Zak, Cabido, Cáceres, & Diaz, 2008; Cáceres,
40 2014). Despite many outstanding features that make these complex ecosystems worthy of
41 protection, the Chaco forest is a poorly represented ecoregion in the Argentinean and South
42 American protected area systems (Izquierdo & Grau, 2008; Mateucci & Camino, 2012).
43 Furthermore, the current legal regulation for the region, which is crucial for generating practices
44 to mitigate the impacts of forest fragmentation, is liberal and permissive, thereby promoting
45 deforestation (Mastangelo & Gavin, 2012; Torrella, Ginzburg, Adámoli, & Galetto, 2013). The
46 generalized expansion of agriculture, driven by global trends in technology and soybean markets

47 (Grau, Gasparri, & Aide, 2005) and by global changes in precipitation regimes (Zak, Cabido,
48 Cáceres, & Diaz, 2008; Hoyos et al., 2013), has promoted the clearing of approximately 6 million
49 ha of native forest over the last three decades (Torrella, Ginzburg, Adámoli, & Galetto, 2013;
50 Grau & Aide. 2008). In particular, the generalized expansion of anthropic land uses are related to
51 the sharp drop of the Gran Chaco natural ecosystems which lead the exiting protected areas to a
52 worrying ecological isolation (Matteucci & Camino, 2012). Although efficient programs to
53 conserve forest biodiversity in fragmented landscapes require a sound understanding of the
54 evolution and spatial distribution of the size of forest fragments over time (Zuidema, Sayer, &
55 Dijkman, 1996), these issues remain almost unknown for the Gran Chaco forests. Regional
56 patterns of forest fragmentation have been recently described in different sectors of the Gran
57 Chaco (e.g., Grau, Gasparri, & Aide, 2005; Hoyos et al., 2013; Zak, Cabido, Cáceres, & Diaz,
58 2008) but fragmentation studies accounting of the relation of forest loss and spatial pattern
59 changes over time are still necessary.

60 Thus, the purpose of this paper is to examine the forest fragmentation of the Gran Chaco
61 over the last 30 years accounting for the interdependencies between forest loss and spatial pattern
62 changes. The research, based on multi-temporal land cover maps (1979, 1999 and 2010) of
63 central Argentina addressed the following questions: (i) How did forest cover change? (ii) How
64 did forest spatial pattern vary? (iii) Which is the relative importance of forest loss and spatial
65 pattern on the fragmentation process?

66 In particular, we quantify forest loss and spatial pattern changes using random sampling
67 techniques on multi-temporal maps followed by a bootstrapping significance test (Fortin,
68 Jacquez, & Shipley, 2012; Manly, 2006). In order to investigate the relationship between forest
69 loss and spatial pattern changes over time we built specific bidimensional ‘relationship spaces’
70 (*sensu* Long, Nelson, & Wulder, 2010) in which the variation of spatial pattern metrics (e.g.

71 Mean Patch Size, Patch Density, Edge Density) were plotted against different levels of forest
72 cover. We assumed that the relative importance of forest loss and spatial pattern varies through
73 space and time. By interpreting the observed forest cover and spatial pattern changes, we
74 contribute to stress the ecological value of the remaining forest patches in order to prioritize
75 conservation efforts in this fragile and highly vulnerable ecosystem.

76

77 **Material and methods**

78 *Study area*

79 The study area is located at the southern extreme of the dry Chaco, to the northeast and
80 northwest of Cordoba Province, in central Argentina (Fig. 1), and it belongs to the Chaco
81 Phytogeographical Province (Cabrera, 1976). Its lowlands were formerly dominated by
82 *Aspidosperma quebracho-blanco* and *Schinopsis lorentzii* subtropical seasonally dry forests
83 (Bonino & Araujo, 2005; Zak & Cabido, 2002). At present, the non-cultivated area is covered
84 mostly with secondary semi-deciduous forests and shrub lands, alternating with patches of old-
85 growth forests and open shrub lands. The plant communities in the arid and semi-arid Chaco of
86 Cordoba are known in detail from the works of Cabido, Acosta, Carranza and Diaz (1992),
87 Cabido, González Albarracin, Acosta and Díaz (1993), Cabido, Manzur, Carranza and Gonzalez
88 Albarracin (1994) and Zak and Cabido (2002). While forest loss and conversion have affected the
89 species richness of both plants and animals, (Cabido, Manzur, Carranza, & Gonzalez Albarracin,
90 1994; Gardner, Cabido, Valladares, & Diaz, 1995), well-conserved forest patches have been
91 reported to comprise the highest alpha diversity in the area (Cagnolo, Valladares, Salvo, Cabido,
92 & Zak, 2008) and to provide more efficient ecosystem services, such as carbon storage (Conti &
93 Díaz, 2013). We sampled a wide area of almost three million hectares in central Argentina that
94 are representative of the Gran Chaco landscape and the main processes that have shaped it over

95 the last decades (Hoyos et al., 2013). The area is organized into three sectors, which are
96 designated as ‘West’ (W), ‘Northwest’ (NW) and ‘Northeast’ (NE) (Fig. 1). The first two sectors
97 are arid plains located on the west of Cordoba mountain range, and the last one occupies the east
98 of the same range (eastern semi-arid plain).

99 The climate is warm temperate to subtropical, with a mean annual temperature ranging from 16
100 °C in the northeast to 19 °C in the northwest and west; the mean annual rainfall decreases in the
101 same direction from more than 800 to 500 mm (Zak, Cabido, Cáceres, & Diaz, 2008). A
102 pronounced water deficit exists in the west of the area (Zak, Cabido, Cáceres, & Diaz, 2008;
103 Hoyos et al., 2013). In the case of the northeast sector, the recent increase in annual precipitation,
104 specifically during the growth period, has made crop production possible and profitable; this was
105 not the case in the west sector, where crop production is not possible without irrigation (Hoyos et
106 al., 2013). Indeed, Zak, Cabido, Cáceres and Diaz (2008) and Hoyos et al. (2013) reported lower
107 deforestation in the NW and W sectors, primarily related to logging for nonagricultural purposes,
108 such as the extraction of firewood and timber and clearing for natural pastures.

109 *Forest cover maps*

110 Forest cover maps were derived from existing large-scale cover maps of the area relative
111 to the years 1979, 1999 and 2010 (Hoyos et al., 2013). Zak and Cabido (2002), comparing remote
112 sensed images and phytosociological field data, found a good correspondence between Landsat
113 TM classification and vegetation types on the Gran Chaco. Thus, in this study the analysis of
114 forest loss and configuration changes was based on land cover maps derived from previous
115 studies (Hoyos et al., 2013). These maps were produced based on Landsat satellite images for the
116 years 1979, 1999 and 2010 and extensive field work for accuracy assessment (Congalton &
117 Green, 1999). To identify the land–cover units, three Landsat MSS scenes from February 1979,
118 three Landsat TM scenes from November 1999, and three Landsat TM scenes from March 2010

119 were used. All Landsat images were acquired during the vegetation growing season of the
120 southern Hemisphere. The classification of Landsat MSS and TM images resulted in reliable
121 land-cover maps (overall accuracy 80%) composed of five vegetation classes: closed forest, open
122 forest, shrublands, halophytic vegetation and cultural vegetation (croplands and urban areas).
123 Further details on the construction of the digital maps, confusion matrices and their accuracy
124 assessment can be found in Hoyos et al. (2013). For our analysis, we selected only closed forests
125 from the other land-cover units. These forests correspond to lowland seasonally dry forests, with
126 *Aspidosperma quebracho-blanco* (white quebracho) and *Schinopsis lorentzii* (red quebracho) as
127 dominant trees and a canopy of at least 50% cover (Hoyos et al., 2013).

128 *Data analysis*

129 We analyzed forest change over time (years 1979, 1999, 2010) in the southern extreme of
130 the Gran Chaco, using a sample based approach which specifically accounts for the
131 interdependencies between landscape composition and configuration changes. The chosen
132 approach offers a sound frame to describe the relation between forest loss and spatial pattern
133 changes (Neel, McGarigal, & Cushman, 2004; Wang, Blanchet, & Koper, 2014) that is crucial
134 for a correct interpretation of the ongoing landscape processes (Gustafson & Parker, 1992;
135 Hargis, Bisonette, & David, 1998, Frate et al., 2014). Moreover, the sample based approach
136 allows producing statistically valid estimates of forest cover and spatial pattern at regional and
137 continental scales (Hassett, Stehman, & Wickham, 2012). The procedure consists of four
138 sequential steps:

139 1- Selection of the fragmentation Pattern Indices (PIs): We selected a set of four non-redundant
140 PIs that have been widely suggested in the literature for fragmentation analysis (Haines-Young &
141 Chopping, 1996; Uuemaa, Antrop, Roosaare, Marja, & Mander, 2009) and that have been

142 previously reported to be adequate for sample-based estimations of landscape pattern (Hassett,
143 Stehman, & Wickham, 2012). In particular, after measuring the percent of the landscape covered
144 by forests (%Forest), we calculated the following spatial pattern metrics: Mean patch size (MPS),
145 Patch Density (PD) and Edge Density (ED). McGarigal and Marks (1995) provided the formula
146 for each of the selected PIs.

147 2- Landscape sampling and PI calculation: A set of non-overlapping square grid units was
148 randomly sampled (totaling 10% of the total extent of each sector), and for each sampling unit,
149 the selected fragmentation PIs were computed using FRAGSTATS (McGarigal & Marks, 1995).
150 To avoid the border effect, only grid cells that were entirely included in the analyzed areas were
151 considered. The analysis was performed at two plot dimensions comparable to those commonly
152 used for local and regional forest monitoring: 1 and 10 square kilometers (Wulder et al., 2008).

153 3- Statistical comparison: The PI estimators (in our case, the arithmetic mean; see Hassett,
154 Stehman, & Wickham, 2012 for details) were calculated, and a bootstrap procedure (Bias-
155 Corrected and accelerated bootstrap) was applied to obtain 95% confidence intervals (Fortin,
156 Jacquez, & Shipley, 2012).

157 4- Trajectory analysis: The temporal trajectory of each sector was identified as follows. For all of
158 the chosen fragmentation PIs, a specific relationship space (*sensu* Long, Nelson, & Wulder,
159 2010) was produced by projecting all of the computed spatial pattern metric values (ED, PD and
160 MP) per sampling plot against the percent of forest cover (%Forest). The construction of a multi-
161 temporal relationship space derived from sampled landscapes offered sound insight for spatial
162 configuration metrics (Long, Nelson, & Wulder, 2010) and was used here in the forest
163 fragmentation analysis. Then, the arithmetic mean of each of the selected PIs was plotted in the
164 relationship space, and the temporal trajectories for each sector (W, NW, NE) were drawn by
165 connecting PIs means chronologically with arrows.

166

167 **Results**

168 The proportion of forest cover in the Gran Chaco declined greatly over time (Fig. 2 and
169 Table 1), and forest loss became particularly striking during the last decade (1999-2010). Thirty
170 years ago, the percent cover of forests was clearly higher in the analyzed sectors. In fact, in 1979,
171 forest cover ranged from 45% of the area in the NW sector, to 37% in the W, to only 19% in the
172 NE. Moreover, while in the first time period (1979-1999), the rate of change in forest cover
173 looked very similar in all sectors (the trajectories in Fig. 2 are almost parallel), in the last time
174 period (1999-2010), it was accelerated for the western sectors (NW and W). In the most recent
175 data, the percent of forest throughout the region was very low, with values of 1.9% for the NE,
176 6.9% for the NW and 5.6% for the W sector.

177 Spatial pattern metrics are characterized by index-specific behaviors in relation to forest
178 cover (Fig. 3 a-c). For instance, as shown by the sample-based relationship space of MPS vs. %
179 Forest (Fig. 3 a), mean patch size tends to be very low for forest cover values below 50%; MPS
180 values exponentially increase when the forest cover exceeds the 50%. Edge density is
181 characterized by a symmetric parabolic relationship with forest cover (Fig. 3 b). ED is low at
182 high and at low forest cover values and presents a positive high peak at intermediate values of
183 forests cover (~ 50). Patch density has an asymmetric parabolic shape along the forest cover
184 gradient (Fig. 3 c). PD is very low for landscapes dominated by forests, tends to increase as the
185 percent of forest cover declines, peaks when forest reaches almost 20% of the landscape area and
186 declines as the forest cover becomes close to 0.

187 The temporal trajectories of each sector (W, NW, NE) into the specific relationship
188 spaces, per 1 km sampling plot, are reported in Fig. 3 a-c. The general trends of PIs for the 10 km
189 plots are similar to those for the 1 km case and are reported in Appendices S1 and S2. In

190 correspondence with the evident decrease in forest cover that occurred in all three sectors, the
191 spatial pattern metrics tended to vary significantly (Table 1). As observed for the forest cover,
192 changes in the spatial pattern parameters occurred at lower rates in the first time period (1979-
193 1999) and accelerated in the second one (1999-2010).

194 The temporal trajectories of MPS for the three sectors (NW, W and NE) were included in the
195 decreasing tile of the relationship curve (forest cover below the 50%). The mean dimension of the
196 forest patches (MPS) in 1979 were largely different in the analyzed sectors and had high values
197 in the NW (45.57), intermediate values in the W (37.18) and small values in the NE (18.46) (Tab.
198 1; Fig 3a). While modest variations in the MPS values were evident in the first time period
199 (1979-1999), the MPS values significantly decreased in the three sectors over the last time period
200 (1999-2010), dropping to 1.66 ha in the NW, 0.71 ha in the W and 0.30 in the NE.

201 The edge density of the forests in the study area significantly decreased over time (Tab. 1; Fig.
202 3b). The projection of multitemporal ED estimators in the specific relationship space portrayed
203 three temporal trajectories which are included in the decreasing left side of the parabolic relation
204 curve. In the Northeast sector, the prior ED value collapsed from 47.50 to 7.10. In the other two
205 sectors, although a significant reduction of ED also occurred, the magnitude of the change was
206 less extensive. ED in the NW sector decreased from 73.63 to 22.87, and in the W sector, it
207 decreased from 70.70 to 23.51. The variation in forest Patch Density (PD) over the last 30 years
208 was less evident (Tab. 1; Fig. 3c). The projection of PD estimators in the specific relationship
209 space portrayed temporal trajectories that move from the right to the left tile of the curve. During
210 the first time period (1979-1999), significant reduction in the density of forest patches occurred
211 on the NW (from 5.86 to 4.66) and NE (from 6.43 to 2.36) sectors, while in the W sector, the PD
212 values remained almost constant (from 6.28 to 6.64). In the second time period, a weak but

213 significant reduction of PD occurred in the NE (from 2.36 to 1.55) and W (from 6.94 to 5.28)
214 sectors, which was less evident and not significant in the NW sector (from 4.30 to 4.34).

215

216 **Discussion**

217 Our results show that the drastic transformation of the Gran Chaco landscape that has
218 occurred over the past decades (Hoyos et al., 2013; Zak, Cabido, Cáceres, & Diaz, 2008) also
219 gave rise to a conspicuous process of forest fragmentation. The statistical comparison of PIs over
220 time not only confirmed the consistent process of forest loss, which was particularly striking in
221 the last decade, but also pinpointed significant changes in the spatial pattern of the remaining
222 forest patches. In this way, we provide clear evidences of the dramatic forest fragmentation that
223 has disrupted the Gran Chaco landscape over the last 30 years. In fact, human activities such as
224 cattle ranching (Caldas, Goodin, Sherwood, Campos Krauer, & Wisely, 2013), firewood
225 extraction (Hoyos et al., 2013) and soybean production (Grau, Gasparri, & Aide, 2005; Hoyos et
226 al., 2013; Mastrangelo & Gavin, 2012; Zak, Cabido, Cáceres, & Diaz, 2008) have shaped the
227 southern Gran Chaco landscape in recent decades and has likely constrained the residual forests
228 into very small and isolated areas.

229 Trajectory analysis underlined differences on the relative importance of forest loss and
230 spatial pattern changes through the analyzed sectors and time steps. The analysis of the forest
231 mean patch size showed similar temporal changes in all of the analyzed sectors and depicted
232 modest variations in the first time step followed by a remarkable process of forest fragmentation
233 in the last decade. The constant value of MPS during the first time step despite the significant
234 loss of forest cover, is most likely related to the removal of patches belonging to different class
235 size categories (from small to very large). Note that forest patches scattered throughout the
236 landscape, may serve as stepping stones, which are crucial for species dispersal enhancing the

237 overall connectivity across the landscape (Saura, Bodin, & Fortin, 2014). Thus, the removal of
238 entire patches, as in this case, probably reduces the chance for organisms to move from a patch of
239 forest to another (Schumaker, 1996), promoting isolation effects and biodiversity loss across the
240 entire landscape (Fahrig, 1997, McGarigal, Cushman, & Regan, 2005). The decrease in patch size
241 which took place during the last decade highlighted a worrying forest fragmentation occurring
242 across the entire landscape. The sharp drop in patch size depicts a landscape with small forest
243 patches, the remnant nuclei of forests. This type of transformation could have important
244 consequences for biological diversity. Previous studies confirm that the reduction of natural
245 forest to a very few small patches threatens wildlife survival by reducing not only habitat
246 availability (Fahrig, 1997; Bennett & Mulongoy, 2006) but also the opportunity for organisms to
247 move across the landscape (Schumaker, 1996). Moreover, the implications of this accelerated
248 loss of large patches may have serious consequences for the biodiversity. In fact, the size of
249 fragments seems to be correlated with species richness: smaller fragments generally host fewer
250 numbers of rare and specialist species, but no differences with respect to the large fragments were
251 reported for generalist common taxa (Cagnolo, Valladares, Salvo, Cabido, & Zak, 2008).

252 The sustained decline of ED values over time indicates the existence of shorter forest edges,
253 which is clearly related to the reduction of forest cover on recent landscapes relative to past ones.
254 When, as in our study, the percent of cover of forests in the prior situation is under 50% and
255 continues to diminish over time, the forest edges also decrease. Many authors agree that small
256 forest patches (<10 ha) are entirely influenced by edge habitats (Kapos, 1989; Zuidema, Sayer, &
257 Dijkam, 1996) that have micro-environmental conditions that differ from those of interior
258 habitats, such as more light availability and lower moisture. The implications of these changes
259 could be dramatic for the conservation of the Gran Chaco native flora and fauna and, in
260 particular, for true forest species (*sensu* Hermy, Honnay, Firbank, Grashof-Bokdam, &

261 Lawesson, 1999). For instance, edges could negatively affect the plant diversity of forests,
262 promoting the decline of rare and specialized true forest species, as has already been observed in
263 Chaco remnant patches (Cagnolo, Cabido, & Valladares, 2006). Furthermore, edge effects may
264 favor the invasion of exotic plant species (Torrella, Ginzburg, Adámoli, & Galetto, 2013), alter
265 plant reproduction and impair the regeneration of various species of the natural flora (Aguilar &
266 Galetto, 2004; Aizen & Feisinger, 1994) and fauna (González, Salvo, & Valladares, 2014; Lopez
267 de Casenave, Pelotto, Caziani, Mermoz, & Protomastro, 1998).

268 The trajectory of forest patch density (PD) showed specific temporal changes in the analyzed
269 sectors and depicted a remarkable process of forest fragmentation over the last three decades. The
270 significant drop in patch density over time observed in the NE sector, pinpoints a severe process
271 of forest loss that has caused the forest patches to be on the brink of disappearing. Indeed, the
272 presence of few small remnant forest patches is one of the most typical features of highly
273 fragmented landscapes (Forman & Godron, 1986; McGarigal, Cushman, & Reagan, 2005). In
274 advanced stages of forest fragmentation many patches are completely removed, increasing
275 isolation (Farhig, 1997) and probably with dramatic consequences for biodiversity (Saura, Bodin,
276 & Fortin 2014). In this case, the ongoing fragmentation process does not depend on the breaking
277 apart of forests, with a consequent rise in the number of patches (Riitters, Wickham, O'Neill,
278 Jones, & Smith, 2000), but is due to a severe reduction of forest cover derived from the removal
279 of whole patches (McGarigal, Cushman, & Regan, 2005). A similar process was observed in the
280 first time step (1979 - 1999) of the NW sector where the significant decrease in patch density is
281 related with a consistent reduction of forest cover and whole patches removal. Instead, the stable
282 number of patches observed during the last time step (1999 - 2010) depicts a landscape where
283 forest loss have occurred without the creation of new patches. In the W sector, the moderate
284 increase of PD values during the first time span, describes a weak process of subdivision.

285 Moreover, during the last decade, the accelerated process of forest loss have promoted the
286 removal of many patches with the consequent diminution of PD values. Ecologically, the
287 increase in the number (or density) of the forest patches, is primarily related to a process of
288 subdivision in the former stages of fragmentation may have negative consequences on native
289 biodiversity (see Fahrig, 2003 for a review). The stable number of patches, depicts a transition
290 between a landscape with few medium-sized forest patches to a more fragmented situation with a
291 comparable number of smaller relict patches. In this case, the possible consequences on
292 biodiversity could be trivial if compared to the effects of the habitat loss (Fahrig, 1997;
293 McGarigal, Cushman, & Regan, 2005). Finally, the drastic reduction in the number of patches, as
294 that observed on the NE sector throughout the last three decades is related to forest disappearance
295 in the advanced stages of forest fragmentation and could be clearly considered an important
296 factor promoting negative effects on species richness and dispersal (Fahrig, 2003).

297 Last but not least, the combined effects of the progressive reduction in forest cover and
298 the alteration of forest spatial pattern could promote local extinctions and lead to negative effects
299 on various levels of the trophic network. In this way, fragmentation effects may disrupt basic
300 ecological processes (Tilman, May, Lheman & Novack, 1994). In relation to habitat loss,
301 previous studies have found lower values of plant species richness (particularly rare plants),
302 which, together with edge effects, suggests a negative impact on native and animal-pollinated
303 plants (Torrella, Ginzburg, Adámoli, & Galetto, 2013).

304

305 **Conclusions and conservation perspectives**

306 The results of this study give rise to an important warning: the Gran Chaco has experienced both
307 serious forest loss and spatial pattern changes, and such processes have been greatly exacerbated
308 during the last decade. In the NE sector, where the increase in annual precipitation made crop

309 production possible and profitable and where deforestation was very intense, forest fragmentation
310 has reached values that signal the possibility of extinction. However, even in the western sectors,
311 where crop production is not possible without irrigation and logging is related to the extraction of
312 fire-wood and timber and to clearing for natural pastures, forests were strikingly fragmented.
313 These results are particularly important, considering the relatively short time span analyzed in
314 this study and because although the fragmentation process has been developing over the last 30
315 years, it has accelerated in the last decade. Our findings sound an alarm for researchers and stake-
316 holders because exceeding a threshold of forest exploitation can lead to the irretrievable loss of
317 biodiversity and function of the Chaco ecosystems.

318 The multitemporal overview of the presence and distribution of large and small patches
319 with different spatial pattern characteristics provides a sound framework to guide the
320 development of an effective conservation strategy that includes fragments of varying sizes. This
321 knowledge is particularly important in the Gran Chaco, where preserving larger fragments could
322 promote the conservation of the overall biodiversity, but small fragments located in highly
323 deforested landscapes could also play a role in the conservation of forest species. The results of
324 this study also provide a basis for identifying the more effective arrangement of fragments and
325 the lower threshold of forest cover that is necessary to mitigate the fragmentation effects.
326 Furthermore, it would be useful to identify a better configuration to preserve the remaining
327 fragments as functional units, which is indispensable information for optimizing conservation
328 measures on fragmented landscapes. Distinguishing habitat spatial pattern changes from forest
329 loss could have important implications for species conservation in Chaco forests. where not all
330 species are equally affected by habitat fragmentation. If, for example, some species go extinct
331 mainly because of habitat loss (such as forest specialists and rare native plant species), the
332 solution is straightforward: habitat conservation and restoration (Fahrig, 2007). Instead, if species

333 are threatened mainly because of the increasing habitat isolation (such as common plant species),
334 the problem may appear less obvious as well as its solution. Probably one of the best
335 management solution is to connect up the 'broken apart' pieces of remaining forests by including
336 new corridors or stepping stones (Saura, Bodin, & Fortin 2014). When forest loss is severe and
337 forest spatial pattern changes are relevant (e.g. NE sector), habitat loss and isolation may
338 seriously compromise the survival of the overall natural forest biodiversity (Fahrig, 1997; Hobbs,
339 2002; Bennett & Mulongoy, 2006;). In extremely fragmented landscapes, conservation efforts
340 must concentrate on both, improving the condition of fragments (e.g., reintroduction of native
341 species, removal of exotic species) in order to ensure their continued persistence, and enhancing
342 the surrounding matrix to reduce threatening processes (Hobbs, 2002). The last resort action in
343 highly fragmented landscapes is to reconstruct habitats using replanting and reintroduction
344 techniques that guarantee the increment of both, available habitats for forest species and
345 connectivity (Saura & Rubio, 2010).

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Table 1. Forest Pattern Indices (PIs) values in the West (W), Northwest (NW) and Northeast (NE) sectors for the years 1979, 1999 and 2010. PIs' arithmetic means are estimated from randomly sampled (1x1 km) tiles along with the 95% confidence intervals obtained by a bootstrap procedure (BCa) are reported.

PIs	Sector	1979			1999			2010		
		Mean	Upper CI	Lower CI	Mean	Upper CI	Lower CI	Mean	Upper CI	Lower CI
% of forest	NE	18.46 ^a	19.64	17.31	9.67 ^b	10.97	8.40	1.85 ^c	2.26	1.47
	NW	45.57 ^a	47.93	43.11	39.31 ^b	42.39	36.20	6.85 ^c	7.97	5.68
	W	37.18 ^a	39.29	35.06	32.09 ^b	34.69	29.44	5.57 ^c	6.33	4.84
Mean Patch Size	NE	5.01 ^a	5.78	4.28	4.37 ^a	5.30	3.47	0.30 ^b	0.38	0.22
	NW	21.79 ^a	24.91	18.81	23.90 ^a	27.22	20.75	1.66 ^b	2.41	1.01
	W	17.59 ^a	19.95	15.26	15.66 ^a	18.19	13.20	0.71 ^b	0.81	0.61
Edge Density	NE	47.50 ^a	49.62	45.37	18.70 ^b	20.62	16.78	7.10 ^c	8.37	5.81
	NW	73.64 ^a	76.58	70.59	56.10 ^b	59.51	52.58	22.87 ^c	25.77	20.08
	W	70.70 ^a	73.02	68.34	61.19 ^b	64.40	57.90	23.51 ^c	26.14	20.97
Patch Density	NE	6.43 ^a	6.70	6.17	2.36 ^b	2.57	2.14	1.55 ^c	1.81	1.29
	NW	5.86 ^a	6.22	5.50	4.30 ^b	4.66	3.92	4.34 ^b	4.81	3.88
	W	6.28 ^a	6.64	5.92	6.94 ^a	7.36	6.51	5.28 ^b	5.76	4.79

^{a, b, c} indicate significant differences in the PIs' arithmetic means between the compared dates

Table 1: Forest Pattern Indices (PIs) values in the West (W), Northwest (NW) and Northeast (NE) sectors for the years 1979, 1999 and 2010. PIs' arithmetic means are estimated from randomly sampled (1x1 km) tiles along with the 95% confidence intervals obtained by a bootstrap procedure (BCa) are reported.

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Figure 1. Location of the Great Chaco (black line), Cordoba Province (white), and the three study sectors (gray shades) in Argentina.

Figure 2. Forest loss over time in the analyzed sectors. Percent of cover mean values estimated from randomly sampled (1x1 km) units (symbol) and the 95% confidence intervals obtained using a bootstrap procedure (BCa) (error bars) are represented.

Figure 3. Trajectories of forests in the relationship space given by the % forest cover and the other fragmentation PIs (MPS: Mean Patch Size (a), ED: Edge Density (b) and PD: Patch Density(c)). Light grey dots represent the observed values of pattern metrics in the entire set of sampled (1x1 km) grid units as symbols (squares, circles and triangles) indicate the arithmetic mean for each sector and date.

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Appendix A. Trajectories of forests in the relationship space given by the % forest cover and the other fragmentation PIs (MPS: Mean Patch Size (a), ED: Edge Density (b) and PD: Patch Density(c)). Light grey dots represent the observed values of pattern metrics in the entire set of sampled (10x10 km) grid units as symbols (squares, circles and triangles) indicate the arithmetic mean for each sector and date.

Appendix B. Forest Pattern Indices (PIs) values in the West (W), Northwest (NW) and Northeast (NE) sectors for the years 1979, 1999 and 2010. PIs' arithmetic means are estimated from randomly sampled (10x10 km) tiles along with the 95% confidence intervals obtained by a bootstrap procedure (BCa) are reported.

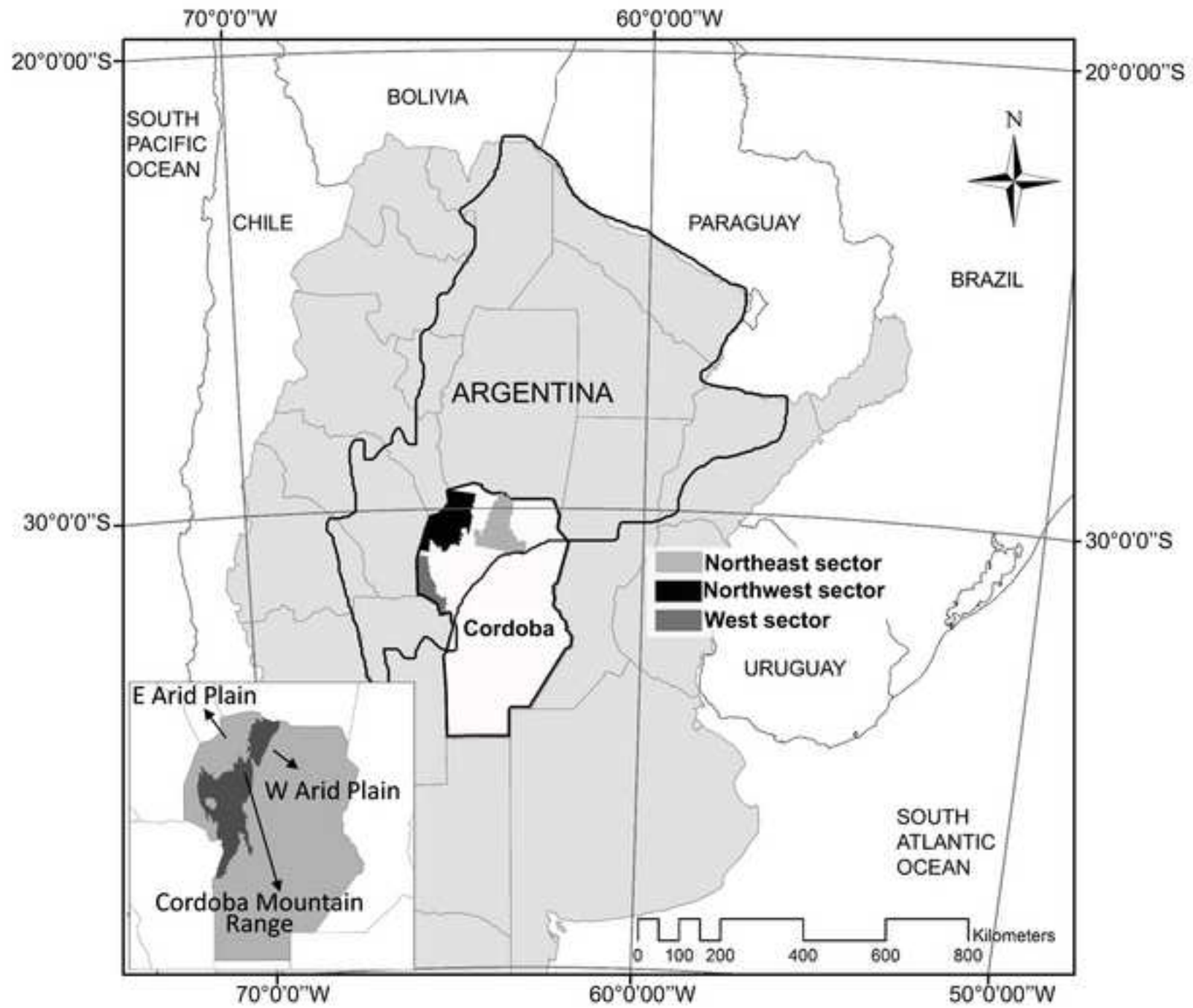
PIs	Sector	1979			1999			2010		
		Mean	Upper CI	Lower CI	Mean	Upper CI	Lower CI	Mean	Upper CI	Lower CI
% of forest	NE	20.27 ^a	23.19	17.41	9.17 ^b	11.77	6.61	1.49 ^c	2.30	0.73
	NW	50.20 ^a	56.07	44.57	47.89 ^a	54.61	41.03	4.46 ^b	6.54	2.59
	W	43.86 ^a	49.92	37.63	30.54 ^b	37.16	23.81	6.24 ^c	7.84	4.67
Mean Patch Size	NE	5.60 ^a	7.46	3.89	6.24 ^a	8.49	4.14	0.37 ^b	0.52	0.22
	NW	90.72 ^a	153.70	33.27	73.25 ^a	118.48	34.90	1.03 ^b	1.35	0.74
	W	140.6 ^a	208.88	74.86	47.46 ^a	97.24	7.16	1.02 ^b	1.25	0.83
Edge Density	NE	51.41 ^a	56.34	46.27	18.28 ^b	22.73	14.07	5.90 ^c	8.83	3.17
	NW	78.83 ^a	85.25	72.27	69.23 ^a	75.94	73.25	17.35 ^b	23.15	11.71
	W	77.19 ^a	82.49	71.94	61.64 ^b	70.32	52.84	26.85 ^c	32.68	20.99
Patch Density	NE	4.96 ^a	5.38	4.53	1.65 ^b	1.97	1.33	1.16 ^b	1.70	0.64
	NW	3.46 ^a	4.20	2.74	2.48 ^{ab}	3.02	1.95	3.22 ^b	4.05	2.40
	W	3.41 ^a	4.18	2.65	4.55 ^b	5.18	3.93	5.28 ^b	6.15	4.38

a, b, c, indicate significant differences on PIs arithmetic means between the compared data

Appendix B. Forest Pattern Indices (PIs) values in the West (W), Northwest (NW) and Northeast (NE) sectors for the years 1979, 1999 and 2010. PIs' arithmetic means are estimated from randomly sampled (10x10 km) tiles along with the 95% confidence intervals obtained by a bootstrap procedure (BCa) are reported.

Figure

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Figure

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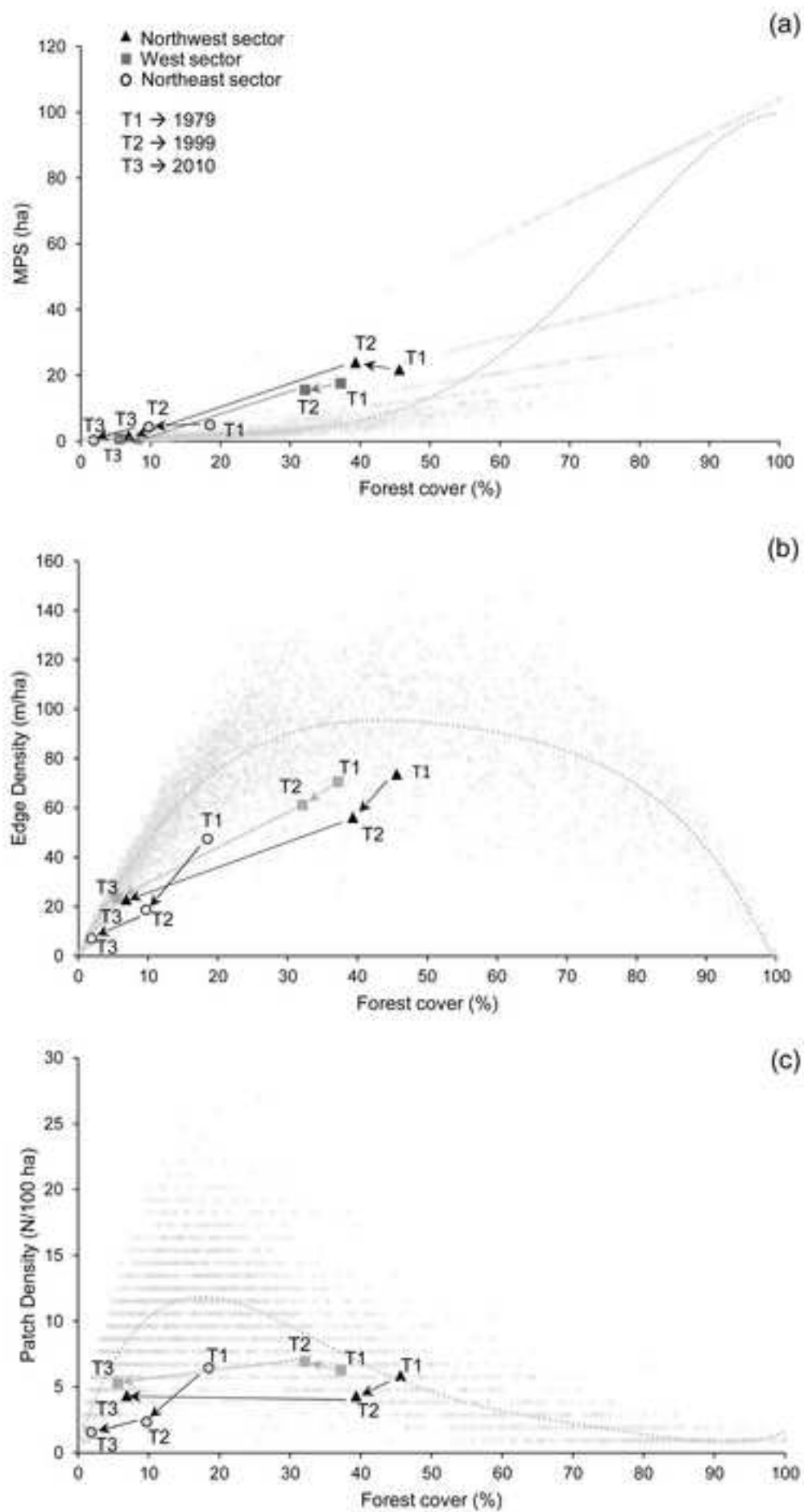
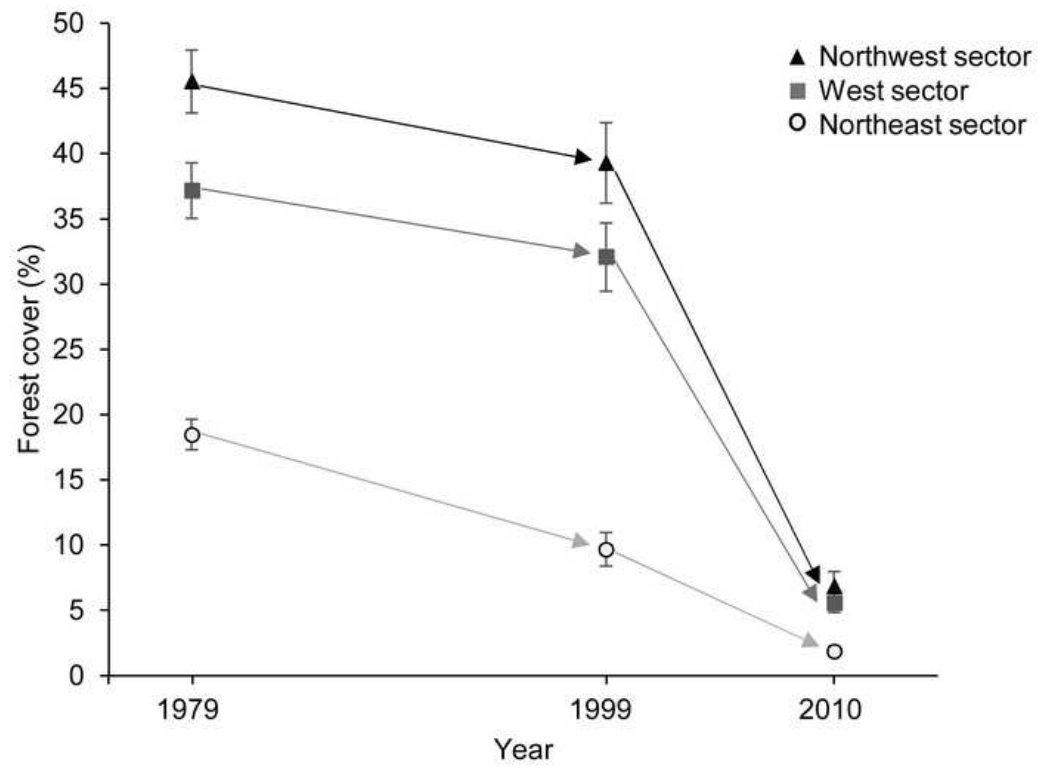


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CAPITOLO 5

FRATE L., CARRANZA M.L., ACOSTA A.T.R., HOYOS L., CABIDO M.

Temporal changes in the fragmentation context of forests at multiple extents: Patch, perforated, edge and interior forests in the Gran Chaco, Central Argentina.

Ecosystems, *Submitted*.

Title:

Temporal changes in the fragmentation context of forests at multiple extents: Patch, perforated, edge and interior forests in the Gran Chaco, Central Argentina

Running head:

Multi-scale pattern of forest fragmentation

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Abstract: The context in which a forest exists, strongly influences forest function and sustainability but this issue remains almost unknown for subtropical ecosystems. In this paper, we quantified the fragmentation context of forest over the last three decades in the dry Chaco (Central Argentina) at multiple scales.

We classified forest locations (pixels) as interior, dominant and patches, based on forest cover (P_f) and connectivity (P_{ff}) values, measured in the forest location surroundings by using a moving window device fixed at eight different extents (from local ~ 6 ha to regional ~ 8300 ha). Specific multi-scale profiles of forest fragmentation (years 1979 and 2010) were defined and compared by representing P_f versus P_{ff} mean values across the selected range of spatial extents.

The dry Chaco, have undergone to an intensive process of forest fragmentation that affected the spatial pattern of forests at different scales and that is more evident at larger ones. Multi-scale fragmentation analysis depicted a landscape where local forest exploitation, that perforates forests cover, coexists with extensive forest loss, which reduces forests to small and isolated patches. Such changes were most likely the result of the interplay between human activities and environmental constrains that have shaped the spatial pattern of forests across a range of scales. Based on our results, the conservation and sustainable management of the dry Chaco should take into account the context of each habitat location and the scales over which forest pattern might be preserved, altered or restored.

Keywords: Landscape Context Analysis, Moving Windows, Conservation, Dry Chaco, Forest Loss, Multi-scale profile

1 **Introduction**

2 The extent of anthropogenic fragmentation of natural forests of many tropical and subtropical
3 ecosystems constitutes one of the most severe causes of biodiversity loss worldwide (Wade and
4 others 2003; Foley and others 2005). Forest fragmentation refers to the amount of forest and its
5 spatial pattern, and both can be measured on raster land-cover maps derived from satellite images
6 (Gustafson 1998; Riitters and others 2002). During forest fragmentation processes, forest cover
7 declines and its spatial pattern varies over time. In a fragmented landscape, a given amount of the
8 remaining forests can be arranged in several manners (i.e., dispersed, clumped, etc.) with different
9 consequences on ecological processes and biodiversity (e.g., Farigh 1997). For instance,
10 fragmentation reduces the extension of interior forests, intended as those forests which, placed in a
11 forest-dominated neighborhoods (Riitters and Wickham 2012), supply the natural habitat to true
12 forest species (Hermly and others 1999; Gonzalez and others 2010; Pellissier and others 2013).
13 Moreover, fragmentation promotes the increment of forest edges through the creation of dispersed
14 holes (perforated pattern) or by the progressive loss of habitat in a wave-like manner, beginning on
15 one edge of the landscape and moving progressively across the landscape (edge pattern) (Forman
16 1995). Thus, fragmentation promotes the expansion of edge habitats characterized by specific
17 environmental and biological conditions (microclimate, vegetation, etc.) that differ from interior
18 forest ones (Levenson 1981; Ries and others 2004; Harper and others 2005). In the severe stages of
19 fragmentation, wide forests can be divided into many small patches where rare and specialist
20 species tend to disappear and replaced by generalists (Bender and others 1998; Matthews and others
21 2014).

22 According to the hierarchical theory (e.g., O'Neill and others 1986), complex landscapes
23 (e.g., heavily affected by human disturbance) do not exist in isolation but they have a hierarchical
24 structure (O'Neill and others 1986; Zurliini and others 2006). In other words, focus landscapes are
25 nested within larger landscapes, that are nested within even larger landscapes and so on (McGarigal

26 and others 2005). Consequently, most of the observed spatial patterns and the ongoing landscape
27 processes take place at specific hierarchical levels and at certain characteristic scales that often
28 depend on how and where land-use policies and management strategies interact with the
29 environment (Zurlini and others 2006). On the other hand, the characteristic scale at which a given
30 process emerges hardly influences the manner in which organisms perceive and use the landscape
31 (e.g., dispersal strategies, habitat quality, habitat suitability, etc.) (McGarigal and others 2005).
32 Such hierarchical nature of the landscape makes single scale studies of little use except for very
33 specific frames of reference (Riitters 2005), whereas it makes the multi-scale analysis imperative
34 for a complete understanding of landscape spatial patterns and processes. An interesting approach
35 for accounting of forest fragmentation at different scales, was proposed by Riitters and others
36 (2000, 2002) that classified forests in four categories of fragmentation based on the amount and
37 connectivity of forests existing in the surrounding landscape (context). According to Riitters and
38 others (2000), given a forest area, it can be classified in patches, perforated, edge and interior
39 forests depending on forest cover and connectivity in a specific context, that can be set and
40 described inside of a range of extents or window sizes ranging from local to regional. The rationale
41 behind this approach is that the context in which a forest exists, strongly influences forest function
42 and sustainability.

43 Natural forests in developing tropical and subtropical countries are among the most
44 threatened ecosystems worldwide (Hoekstra and others 2005). Major dry forests are seriously
45 exposed to agricultural clearing and timber extraction, both authorized and illegal, and to forest fires
46 (Southworth and Tucker 2001; Carr and others 2009; Honey-Rosés 2009). The Gran Chaco, which
47 is among the largest seasonally dry subtropical forests in the world occurring in Argentina,
48 Paraguay and Bolivia (Bucher 1982; Zak and others 2008; Cáceres 2014), have been cleared for
49 agriculture and, nowadays, has been replaced by a mosaic of crops, secondary forests and remnants
50 of primary forests (Gasparri and Grau 2009; Hoyos and others 2013). Despite the Gran Chaco is
51 one of the most threatened ecosystems in Latin America (Grau and Aide 2008), Chaco forests are

52 poorly represented in the Argentinean and South American protected area systems (Izquierdo and
53 Grau 2008; Mateucci and Camino 2012). Furthermore, the current legal regulation for the region,
54 which is crucial for generating practices to mitigate the impacts of forest fragmentation, is liberal
55 and permissive, thereby promoting deforestation (Mastrangelo and Gavin 2012; Torrella and others
56 2013). It is widely known that efficient programs to conserve and manage forests in fragmented
57 landscapes must account of both, the spatial characteristics of forest patches in the landscape (e.g.,
58 size, shape, connectedness) as well as the nature of the forests' surroundings (context) over a range
59 of scales (e.g., Zaccarelli and others 2008; Townsend and others 2009). Regional patterns of
60 ecosystem fragmentation have been recently described in different sectors of the Gran Chaco (e.g.,
61 Zak and Cabido 2002; Gasparri and Grau 2009; Hoyos and others 2013; Carranza and others 2014)
62 but, fragmentation studies that take into account of forest context and the multi-scale nature of
63 forest fragmentation are urgently needed (Matteucci and Camino 2012). An interesting point to
64 explore is whether the fragmentation context of forests in the dry Chaco changes across scales
65 pinpointing specific processes of landscape transformation. Thus, how forests are modified
66 (interior, perforated, edge, patch) in a specific context represents a further point to investigate. For
67 instance, transitions in forest dominance across scales (interior, perforated, edge, patch) could
68 indicate a landscape where different types of fragmentation (local and regional) coexists. On the
69 other hand, the presence of patchy forests at all scales, might be related with late-stage
70 fragmentation characterized by tinny patches dispersed in a non-forest matrix. Finally, the
71 dominance across scales of interior forests, is likely related with non fragmented and well preserved
72 landscapes.

73 In this research, we extended the Riitters and others (2000) fragmentation context analysis
74 into the temporal domain (the last 30 years) to report the effects of landscape change on the spatial
75 pattern of forests at multiple scales of the Argentinean Dry Chaco. We assumed that the influence
76 of fragmentation over time on forest cover and connectivity varies across scales, from local to
77 regional. By identifying the multi-scale behavior of forest fragmentation context, we contribute to

78 deeply describe how fragmentation occurs and we offer the basis to formulate scientifically sound
79 hypothesis about the mechanisms driving forest changes. The accurate description of the multi-scale
80 nature of forest fragmentation has a great potential for planning and managing landscape
81 fragmentation across scales with a predictable effect on biodiversity.

82 In order to provide a complete understanding of the Riitters and others (2000) multi-scale approach
83 here adopted and to supply a frame to interpret the behavior of real fragmentation patterns over
84 time, we first exercised the procedure on neutral simulated landscapes. Modeled maps represent a
85 variety of situations ranging from landscapes where forests are scattered and weakly connected to
86 landscapes characterized by wide and highly connected forests (Gardner and others 1987).

87

88 **Materials and Methods**

89 *Study area*

90 The study area is located at the southern extreme of the dry Chaco, to the northeast and northwest of
91 Cordoba Province, in central Argentina (Figure 1a). The area (~3 million of hectares) was divided
92 into three sectors based on a combination of administrative boundaries, vegetation units and
93 environmental characteristics, denominated as "Northeast" (NE), "Northwest" (NW) and "West"
94 (W). The first sector is located east of a mountain range (eastern semi-arid plain), while the other
95 two occupy the western arid plain, west of the same range. The climate is warm temperate to
96 subtropical, with a mean annual temperature ranging from 16 °C in the northeast to 19 °C in the
97 northwest and west; the mean annual rainfall decreases in the same direction from more than 800 to
98 500 mm (Zak and others 2008). A pronounced water deficit exists in the north and west of the area
99 (Zak and others 2008; Hoyos and others 2013). The area belongs to the Chaco Phytogeographical
100 Province (Cabrera 1976): its lowlands were formerly dominated by *Aspidosperma quebracho-*
101 *blanco* (white quebracho) and *Schinopsis lorentzii* (red quebracho) subtropical seasonal forests
102 (Cabido and others 1992; Cabido and others 1994; Zak and Cabido 2002). During the last 30 years

103 the three sectors have experienced a net forest loss of approximately 615000 ha (Figure 1c) which is
104 the ~ 60% of the initial cover. This percentage was heterogeneously distributed across the three
105 sectors. Northern sectors (Northeast and Northwest) had experienced the major exploitation with a
106 net forest loss of 79% and 66% respectively in the last 30 years whereas in the West sector this
107 value amounted to 37% (Hoyos and others 2013).

108 *Forest cover Maps*

109 Forest cover maps were derived from existing large-scale cover maps of the area relative to the
110 years 1979 and 2010 (Hoyos and others 2013). These maps were produced based on Landsat
111 satellite images for the years 1979 and 2010 and extensive field work for accuracy assessment
112 (Congalton and Green 1999). Zak and Cabido (2002), comparing remote sensed images and
113 phytosociological field data, found a good correspondence between Landsat TM classification and
114 vegetation types on the Gran Chaco. Thus, in this study the analysis of fragmentation was based on
115 land-cover maps derived from Landsat images (Hoyos and others 2013). To identify the land-cover
116 units, three Landsat MSS scenes from February 1979 and three Landsat TM scenes from March
117 2010 were used. All Landsat images were acquired during the vegetation growing season of the
118 southern Hemisphere. The obtained cover maps showed an overall accuracy of approximately 89%
119 (Kappa statistic = 0.87) for the 2010 images. The spatial resolution of the maps was harmonized to
120 the coarser map (1979) which has a pixel dimension of 79 x 79 m. Eight land-cover units were
121 mapped: closed forest, open forest, closed shrubland, open shrubland, grassland with scattered
122 shrubs, halophytic vegetation, salt, and cultural vegetation. Further details on the construction of
123 digital maps, confusion matrices and their accuracy assessment can be found in Hoyos and others
124 (2013). For our purposes, we aggregated the original land-cover types to focus on the pattern of
125 forest versus non-forest land cover. Closed forest and open forest were grouped into one forest
126 class; the remaining classes were grouped into one non-forest class (Figures 1b, 1d).

127 *The fragmentation Model*

128 Consider a binary map showing pixels of forest and non-forest, and let composition refers to the
129 amount of forested area and configuration to its spatial arrangement or connectivity, we described
130 the multi-scale pattern of forest fragmentation using a moving window device. In particular, the
131 moving window operates by moving a fixed-area window over the map one pixel at a time,
132 calculating selected metrics within the window and returning that value to the center pixel
133 (McGarigal and Cushman 2005). The result is a continuous surface that reflects the context of each
134 forest pixel in neighboring areas. In particular, for each forest pixel, we measured the composition
135 (the probability of finding other pixels of forest- P_f) and connectivity (the probability that a forest
136 pixel is adjacent to another forest pixel - P_{ff}) within a fixed window size. The simultaneous analysis
137 of P_f and P_{ff} values in a specific relationship space enables to depict a wide range of forest spatial
138 patterns (patches, interior, perforated and edges) (see Riitters and others 2002 and; Zurlini and
139 others 2007 for details). When the amount of forest P_f is above a certain threshold, it can be stated
140 that forests dominates the landscape (Riitters and others 2002). According with the percolation
141 theory, for a binary random map, the threshold value is about 0.6 and below it, forests are
142 distributed in “patches” embedded in a landscape dominated by non-forest areas. When the amount
143 of forests is >0.9 we refer to as “interior” forests. For other “dominated by forest” landscapes ($0.6 <$
144 $P_f < 0.9$) we can define a gradient ranging from perforated to edge forests. In particular, if $P_{ff} < P_f$, the
145 fragmentation can be said to be “perforated” (i.e., compact forest cluster that has holes created by
146 small non-forest gaps); conversely, when $P_{ff} > P_f$ the fragmentation can be said to be “edge” (i.e.,
147 compact forest clusters appear next to compact non-forest cluster - Figure 2).

148 *Neutral simulated landscapes*

149 In order to provide a complete understanding of the fragmentation model here adopted we exercised
150 the model with neutral simulated landscapes (Gardner and others 1987). By NLMs (Neutral
151 Simulation Models) it is possible to produce a rich array of spatial patterns describing variations on
152 landscape composition (the amount of the focal land-cover area P_f - Proportion of forest) and

153 connectivity (degree of spatial autocorrelation among adjacent cells H). NLMs may be used as a
154 “laboratory” to manipulate a surprisingly set of real-like spatial patterns resulting from
155 fragmentation (e.g., With and King 1997; Zurlini and others 2007; Frate and Carranza 2013), and
156 thus, providing a baseline to understand and compare the spatial pattern of real landscapes, as in our
157 case the Chaco forests change over the last 30 years. We generated 25 landscapes (appendix I)
158 exemplifying the pattern transition space (Zurlini and others 2014) with different combinations of
159 forest abundance ($0.05 \leq P_f \leq 0.95$) and spatial autocorrelation ($0 \leq H \leq 100$) using the public
160 domain software Qrule, freely available on line (<http://www.al.umces.edu/faculty/bobgardner.html>;
161 Gardner 1999). In general, at similar P_f values, there is a transition from high to low fragmented
162 landscapes with increasing H and from salt and pepper distributed to sharply defined forest patches
163 from top to down (Appendix I).

164 *Multi-scale analysis*

165 Context fragmentation analysis was performed on both the observed forest cover dataset (three
166 sectors and two dates 1979-2010) and the NLM simulated dataset as follows. For each forest pixel
167 in the map, the proportion (P_f) and the connectivity (P_{ff}) of forests were measured at eight spatial
168 extents, using square moving windows of 3 x 3, 5 x 5, 9 x 9, 15 x 15, 25 x 25, 45 x 45, 75 x 75 and
169 115 x 115, corresponding respectively to 5.62 ha, 15.60 ha, 50.55 ha, 140.42 ha, 390.06 ha, 1263.80
170 ha, 3510.56 ha and 8253.72 ha referred to real landscapes. According to Riitters and Wickham
171 (2012), selected window sizes represent several orders of magnitude of spatial scales able to tune up
172 from fine-scale fragmentation processes to coarse-scale ones.

173 For each real and simulated landscape (sectors: NE, NW, E; dates: 1979, 2010 and NLMs), specific
174 multi-scale profiles of forest fragmentation were defined by representing P_f versus P_{ff} mean values
175 across the selected range of spatial extents. Multi-scale profiles were then compared in order to
176 describe the fragmentation processes emerging at different scales and to interpret local, medium and
177 regional scale changes occurred in the dry Chaco thorough the last 30 years.

178 **Results**

179 *Fragmentation analysis on simulated landscapes*

180 Multi-scale fragmentation profiles of real (three sectors: NE, NW, E; two dates: 1979, 2010) and
181 four simulated landscapes are reported in Figure 3 (for all the profiles see the appendix I). We can
182 identify two extreme profiles derived from simulated landscapes here represented as “a” and “b”
183 profiles. The “a” profile is a nearly horizontal line given by constant high $P_f (>0.9)$ and increasing
184 P_{ff} values across extents ($P_{ff} \sim 0.66$ from to ~ 0.99). The multi-scale profile of type “a” describes a
185 landscape dominated by interior forests at all the contexts of analysis, and is characteristic of non-
186 fragmented landscapes with a dominant forest matrix. The “b” profile, is a nearly vertical line
187 where forest cover strongly decreases from small to wide scales ($P_f \sim 0.44$ from to ~ 0.07) and
188 connectivity keeps similar low values ($P_{ff} \sim 0.4$). Type “b” profiles are typical of highly fragmented
189 landscapes where many tinny patches are dispersed in a non-forest matrix. Most of the remaining
190 multi-scale profiles are curves where forests cover (P_f) tend to decrease (dominant to patch forests)
191 and connectivity (P_{ff}) to increase (from perforated to edge) from small to wide scales (e.g. profiles
192 “c” and “d” Fig. 3). Particularly interesting are those profiles that describe different aspects of forest
193 pattern across scales. For example multi-scale profiles that, at smaller extents, are dominated by
194 interior forest and, at medium and large extents, abruptly drop to patchy forests (“c”) describe a
195 landscape where compact forests nuclei (detected at small extents) are embedded in a wide non-
196 forest matrix (detectable only at wider scales).

197 *Fragmentation analysis in the dry Chaco*

198 Multi-scale profiles describing Chaco forests in the compared data depict the presence of
199 perforated, edge and patch forests (Figure 3). Note that all the compared profiles fall under the
200 threshold of $P_f=0.9$ and interior-like profiles are absent. Multi-scale profiles relative to the West
201 sector indicate moderate changes in forest spatial pattern from 1979 to 2010. According with the
202 multi-scale profiles, in the oldest data, the landscape was dominated by forests at all spatial extents.

203 Mean P_f values in the year 1979 ranged from 0.87 at smaller extents to 0.65 at larger ones as
204 connectivity (P_{ff}) from 0.55 to 0.86. P_f and P_{ff} values for the 2010 landscape decreased across all
205 scales. In the last data, P_f declined of 0.04 at small scales and of 0.22 at large scales. Unlike of the
206 1979, the 2010 multi-scale profile shows a shift from forest dominated landscape to patchy pattern
207 over medium-large scales (45 x 45 window, 1263.80 ha)

208 The multi-scale profile of the Northeast sector depicts a highly fragmented landscape in both dates
209 with dominant-perforated forests detectable only at the smallest window ($P_f = 0.61$, $P_{ff} = 0.60$ for
210 1979 and $P_f = 0.61$, $P_{ff} = 0.48$ for 2010). Major changes have occurred in terms of connectivity (P_{ff})
211 that decreased of approximately 0.15 across all the analyzed scales. Such diminution had led to a
212 more dispersed forest pattern.

213 Multi-scale profiles relative to the Northwest sector show considerable changes on forest pattern
214 from 1979 to 2010. At small and medium spatial extents, the 1979 landscape resulted dominated by
215 forests (perforated and edge; P_{ff} from 0.80 to 0.63 and P_{ff} from 0.57 to 0.78) while at large scale
216 (over the 45 x 45 window) forest looked distributed in patches. In the more recent data forest
217 pattern have evolved towards a patchy distribution across the different scales. In particular, forest
218 loss was more severe at larger scales (P_f loss ~ 0.53 at the widest scale).

219 **Discussions**

220 During the last 30 years, the dry Chaco, have undergone to an intensive process of forest
221 fragmentation that affected the spatial pattern of forests at different scales. Multi-scale profiles
222 depict the existence of fragmented forests already in the 70's and an escalation of the fragmentation
223 process in the last 30 years. Furthermore, the multi-scale fragmentation experienced by Chaco
224 forests, depicts a landscape where local forest exploitation, that perforates forests cover, coexists
225 with extensive forest loss, which reduces forests to small and isolated patches. The observed forest
226 pattern across scales could be related with different agriculture and forest logging practices

227 (Steininger and others 2001; Alves, 2002; Geist and Lambin 2002) as well as with heterogeneous
228 land use policies at different administrative levels (Cáceres 2014).

229 In particular, in the W sector, the diminution over time of forest cover and connectivity at different
230 scales depict a relative increase of forest fragmentation. Forest loss at local scale were in the 70's
231 and is still related with forest perforation processes. Such pattern is most likely related with local
232 forest utilization, cattle grazing activities and traditional agricultural practices that has characterized
233 this sector over time (Zak and others 2004, 2008; Hoyos and others 2013). Here, land exploitation is
234 probably constrained by the harsh environmental condition (modest rainfalls and clayey and salty
235 soils) that have restricted the development of extensive agriculture. Instead, the patchy distribution
236 observed in the recent data at larger scales suggests that forest loss has been a local-scale
237 phenomenon that accumulated into a broad-scale effect (Wickham and others 2008) because it has
238 been pervasive and not limited to locations where forests was not dominant. The general pattern of
239 fragmentation in the W sector is similar to that observed by Gasparri and Grau (2009) on the
240 northern Arid Chaco, where, environmental conditions, allow the diffused presence of small
241 agriculture parcels and agribusiness deforestation characterized by large clearing events (Geist and
242 Lambin 2002) are not possible.

243 The NE sector showed the most dramatic situation. Having been more than half deforested by 1979,
244 the NE sector, was already a site in an advanced stage of fragmentation. However, it continued to
245 experience a significant fragmentation process during the last decades at all spatial scales. In this
246 sector the continuous loss of large scale forested areas, is most likely related with the combination
247 of good soil conditions (Hoyos and others 2013) and an increase in annual precipitations recorded
248 during the last half century (Zak and others 2004, 2008) that have promoted the NE sector as a more
249 suitable area for agricultural activities. Indeed, the expansion of intensive agriculture driven by
250 global trends in technology (Grau and others 2005) have substituted wide forested areas changing
251 its natural landscape setting.

252 The multi-scale profiles of the NW sector, underline a quick transition of the landscape, from
253 perforated to patchy pattern. The temporal modification of forests in the NW sector depicts a
254 landscape where forest loss have changed from local forest harvesting and clearing practices to
255 regional extraction of large forested areas. As the observed pattern in the 1979 landscape is most
256 likely related with local exploitation of natural resources, the 2010 forest pattern may be the result
257 of the expansion of large-scale productive activities triggered by the significant increase in mean
258 annual precipitations (Hoyos and others 2013).

259 Our results suggest that, in the Gran Chaco, forest fragmentation occurs from small to wide
260 extents and tends to be more severe at larger ones. Therefore, spatially extensive forests are
261 becoming very rare or they are disappearing (especially in the NW and NE sector). Such changes
262 will likely affect the conditions of the forests themselves (Mladenoff and others 1993; Foster and
263 others 1998; Weathers and others 2001; Harper and others 2005) and the ability of forests to supply
264 many ecosystem services as climate regulation (Matteucci and Camino 2012), material provisioning
265 (Cáceres 2014) and food supply (De Marco and Coelho 2004). Furthermore, our findings emphasize
266 the importance of considering the landscape context at multiple scales, which may affect
267 conservation efforts and the sustainable management of dry Chaco ecosystems. Indeed, the nature
268 of surrounding landscapes may strongly alter the quality and the resilience of the forest remnants
269 (Zaccarelli and others 2008; Mairota and others 2015). For instance, the drop below the critical
270 percolation threshold (~ 0.60) of forest cover in the recent data and at larger extents, pinpointed a
271 worrying situation. Indeed, in landscapes near to the critical threshold of fragmentation, minor
272 forest loss can dramatically affect the number of patches, the size of the largest patch and the
273 connectivity and quality of forests remnants (Riitters and others 2002; Saura and others 2014;
274 Mairota and others 2015). The observed reduction of dominant forests (interior and edge forests)
275 could have serious consequences on local biodiversity of the Gran Chaco. In effect, as large extent
276 forests are necessary for maintaining rare and specialist native species (Cagnolo and others 2006),
277 the transition towards a non-forested matrix could promote processes of local extinction. The

278 presence of a patchy pattern at all scales could have serious consequences on the quality of forest
279 remnants. Small patches, embedded in a non-forest matrix ($P_f < 0.6$), are particularly exposed to the
280 influence of edges (Kapos 1989; Zuidema and others 1996) which could negatively affect the plant
281 diversity of forests (Cagnolo and others 2006). Furthermore, edge effects may favor the invasion of
282 exotic plant species (Aizen & Feisinger 1994; Torrella and others 2013), alter plant reproduction
283 and impair the regeneration of various species of the natural flora (Aguilar and Galetto 2004) and
284 fauna (Lopez de Casenave and others 1998).

285 **Conservation Implications and Conclusions**

286 The conservation and sustainable management of Chaco forests requires adequate policies and
287 interventions that account of both, the natural habitats *per se* and the quality of the habitat
288 surroundings (context). Unfortunately, in most Latin American countries, these information are
289 lacking because natural forest surroundings are ignored and the sustainable management of forest
290 context is not perceived as a conservation tool (Matteucci and Camino 2013). Furthermore,
291 protected areas in the Dry Chaco are inadequate and their institution has been mainly based on
292 contingent opportunities rather than on a regional planning process (Burkart 2007). Conservation
293 planners have become increasingly interested in preserving and restoring high quality habitats
294 (sources), particularly where rare threatened, or endangered species are concerned (Lambeck and
295 Hobbs 2002). Based on our results, any conservation measure should take into account the context
296 of each habitat location and the scales over which forest pattern might be preserved, altered or
297 restored to achieve specific goals. For example, one strategy for preserving habitat quality, is to
298 preserve the dominant conditions over the range of scales, or to "fill" perforated areas expanding
299 interior forests. Another conservation strategy could be devoted to that landscapes which host a
300 patchy forest pattern. Forest patches could play an important role in the conservation of forest
301 species because they may function as stepping stones facilitating dispersal among isolated habitat

302 areas (Saura and others 2014). The conservation value of these patches may be improved through
303 the management of the surrounding landscape, i.e. increasing small-scale interior forests.

304 The multi-scale analysis of the dry Chaco has given us the evidence of how fragmentation affected
305 the pattern of forests at all the spatial extents we considered. The observed change is most likely
306 derived from the interplay between human activities and environmental characteristics that have
307 shaped the spatial pattern of forests across a range of scales (from local to regional). Indeed, in the
308 Gran Chaco, decisions related to forests conversion are typically made at local scales (Cáceres
309 2014) as the expansion of industrial agriculture and the large-scale forest clearing events, are
310 mainly triggered by regional and global market trends (Grau and others 2005). If decisions about
311 landscape management and forest conversion continue to be taken ignoring the multi-scale context
312 of forest fragments, huge impacts on ecological functions of the Chaco forests, would inevitably
313 occur over a range of spatial scales. In any conservation planning process, important questions arise
314 when management priorities need to be set. Based on our results, much emphasis should be given to
315 perforated forests and to forests with a cover near to the critical fragmentation threshold. Indeed, if
316 on perforated landscapes, the process of forest loss persists over time, the small-scale perforation
317 might expand and coalesce, giving rise to a new landscape setting dominated by large scale
318 deforested areas.

319

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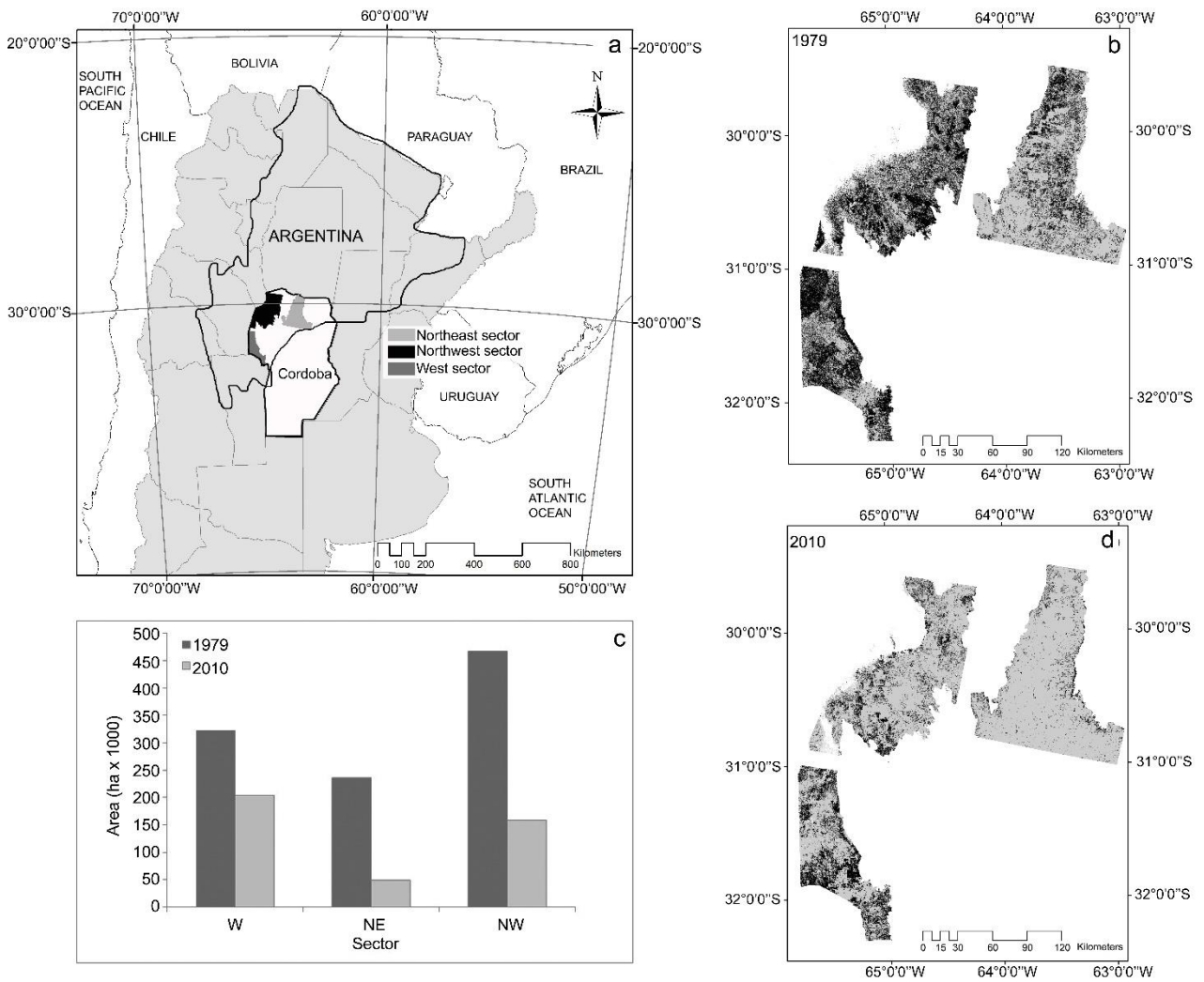
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499 **Figures**

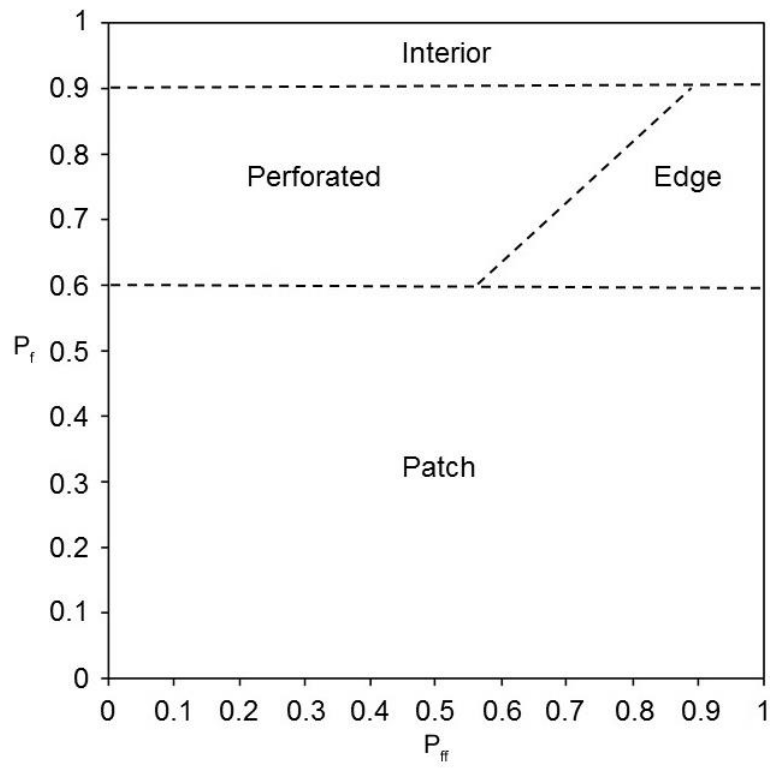
500 **Figure 1.**



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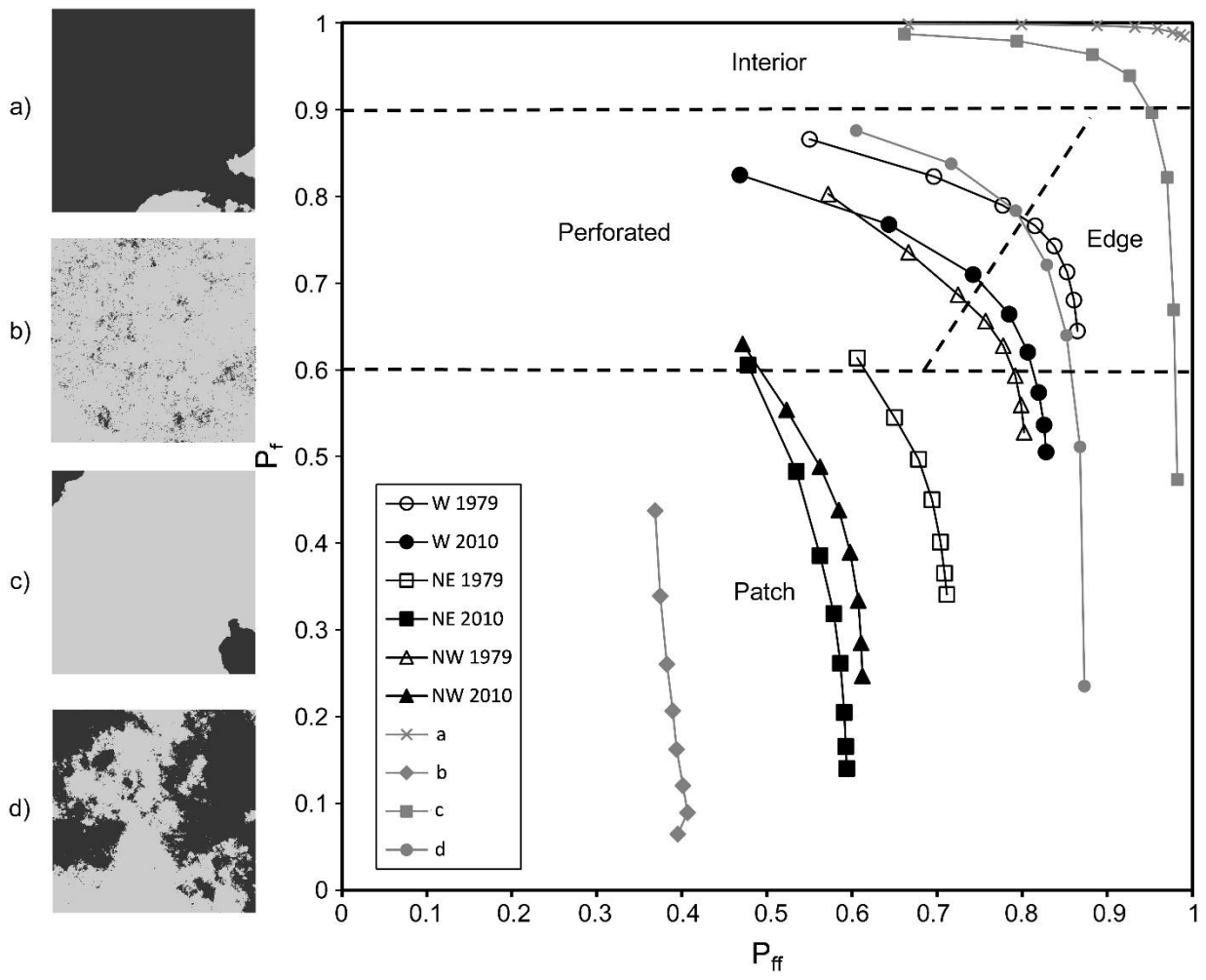
503 Figure 2.



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506 Figure 3.



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509 **Figure captions**

510 **Figure 1.** (a) Location of the Great Chaco (black line), Cordoba Province (white), and the three
511 study sectors (gray shades) in Argentina. (b) Forests distribution in 1979 and (d) Forests distribution
512 in 2010: the binary classification separates forests (black) from non-forests (light grey); (c) Change
513 in forest cover calculated for the entire landscape between 1979 and 2010. W = west sector; NE =
514 northeast sector; NW = northwest sector.

515

516 **Figure 2.** The model used to characterize multi-scale forest fragmentation. P_f and P_{ff} refer to the
517 amount and the connectivity of forest, respectively. Regions of the parameter space corresponding
518 to “interior”, “perforated”, “edge” and “patch” components are marked. (Modified after Riitters and
519 others 2002).

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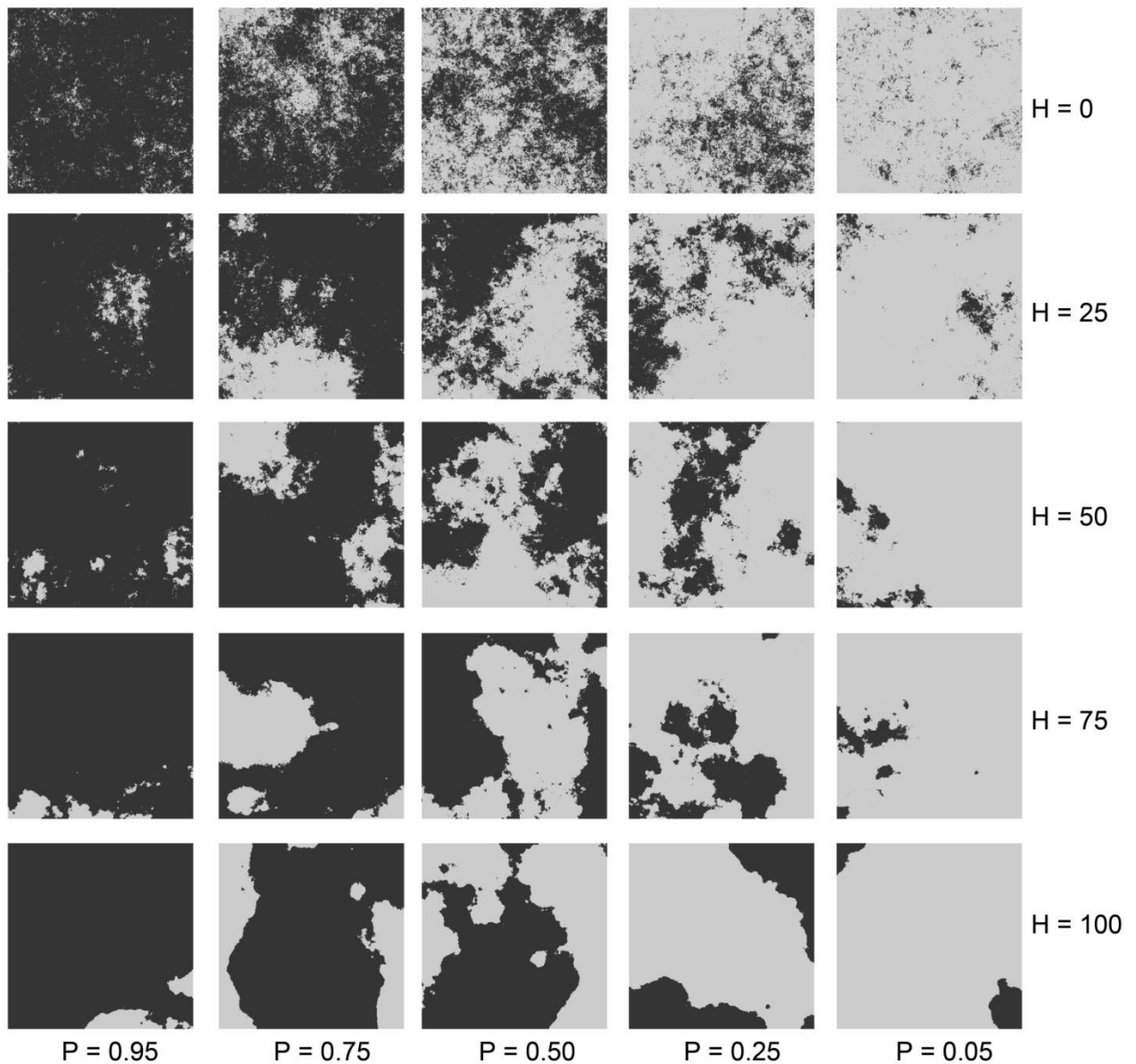
521 **Figure 3.** Multi-scale profiles of real (in black - three sectors: NE, NW, E and two dates: 1979,
522 2010) and simulated landscapes (in light grey) describing forest fragmentation. P_f is the proportion
523 of forests in the analyzed extent and P_{ff} is the connectivity. "a", "b", "c" and "d" are four examples
524 derived from multi-scalar analysis of binary neutral landscapes (forest in black and non forest in
525 grey). a) forest dominated landscape ($P_f \sim 0.95$; $P_{ff} \sim 0.66$ from to ~ 0.99); b) highly fragmented
526 landscape with dispersed small patches ($P_f \sim 0.44$ to 0.07 ; $P_{ff} \sim 0.4$); c) highly fragmented landscape
527 with clumped forests ($P_f \sim 0.98$ to ~ 0.47 ; $P_{ff} \sim 0.66$ to ~ 0.98) and d) intermediate fragmentation
528 level ($P_f \sim 0.87$ to ~ 0.23 ; $P_{ff} \sim 0.60$ to ~ 0.87).

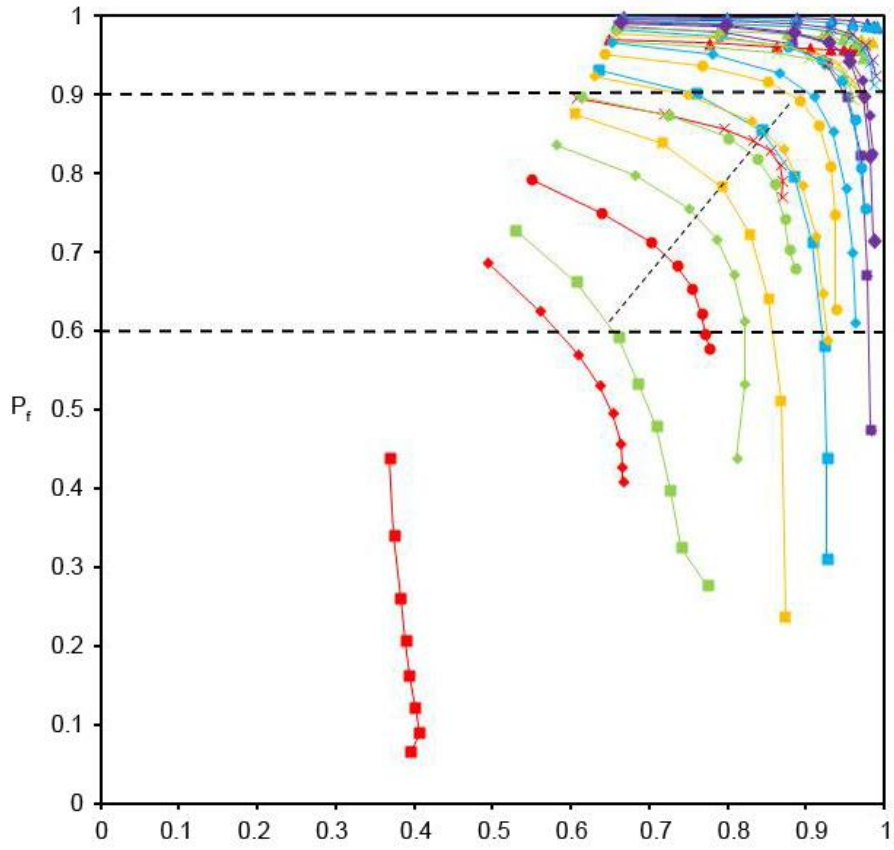
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530 **Appendices**

531 Appendix I.

532 Twenty-five simulated binary maps (forest: black – no forest: light grey) with different
533 combinations of forest abundance ($0.05 \leq P \leq 0.95$) and spatial autocorrelation ($0 \leq H \leq 100$) along
534 with the relative multi-scale profiles. Simulations were performed using the public domain software
535 Qrule (<http://www.al.umces.edu/faculty/bobgardner.html>; Gardner 1999).





- H=0; Pf=0.05
 ◆ H=0; Pf=0.25
 ● H=0; Pf=0.50
 × H=0; Pf=0.75
 ▲ H=0; Pf=0.95
- H=25; Pf=0.05
 ◆ H=25; Pf=0.25
 ● H=25; Pf=0.50
 × H=25; Pf=0.75
 ▲ H=25; Pf=0.95
- H=50; Pf=0.05
 ◆ H=50; Pf=0.25
 ● H=50; Pf=0.50
 × H=50; Pf=0.75
 ▲ H=50; Pf=0.95
- H=75; Pf=0.05
 ◆ H=75; Pf=0.25
 ● H=75; Pf=0.50
 × H=75; Pf=0.75
 ▲ H=75; Pf=0.95
- H=100; Pf=0.05
 ◆ H=100; Pf=0.25
 ● H=100; Pf=0.50
 × H=100; Pf=0.75
 ▲ H=100; Pf=0.95

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SEZIONE III.

Applying Landscape Ecology in Biological Conservation

CAPITOLO 6

CARRANZA M.L., FRATE L., PAURA B. 2012. Structure, Ecology and Plant Richness Patterns in Fragmented Beech Forests. *Plant Ecology and Diversity* 5: 541-551. doi: <http://dx.doi.org/10.1080/17550874.2012.740509>

Structure, ecology and plant richness patterns in fragmented beech forests

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Background: Landscape fragmentation constitutes one of the most severe causes of global biodiversity loss.

Aims: We studied *Fagus sylvatica* forests with different levels of fragmentation to address the following question: do fragmented and non-fragmented forests present a similar floristic composition and richness, structural parameters and ecological features?

Methods: Vascular plant species were randomly sampled based on a beech forest map classified into three fragmentation levels. We compared overall native and diagnostic species richness patterns of the different fragmentation levels using rarefaction curves and the ratio between diagnostic and all species curves. We also contrasted different fragmentation levels of beech forests, focusing on floristic information, structural parameters, standard ecological features and the distribution of edge and clearing species.

Results: Rarefaction analysis showed two opposite trends: the diversity of diagnostic species decreased in fragmented forests as the overall diversity increased. In highly fragmented forests, we found significantly higher values for therophyte and phanerophyte frequencies, light Ellenberg indicator values and edge and clearing species diversity.

Conclusions: The integration of floristic analysis, particularly of certain diagnostic groups, with structural and ecological studies is more sensitive and significant than species richness alone, and could offer useful information for forest conservation and management.

Keywords: Apennine beech forests with *Taxus* and *Ilex* (92/43/EEC - 9210*); diagnostic species; edge and clearing species; Ellenberg indicator values; forest conservation; Habitat Directive 92/43/EEC; life forms; rarefaction curves

Introduction

The extent of anthropogenic fragmentation of natural forests of many tropical and temperate ecosystems constitutes one of the most severe causes of biodiversity loss worldwide (Wade et al. 2003; Foley et al. 2005), and for this reason, it is a primary issue of interest in conservation biology (Meffe and Carroll 1997; Trombulak et al. 2004). Usually, the effects on biodiversity of forest fragmentation are thought to be negative (Franklin et al. 2002; Wade et al. 2003) and long-lasting (Turner 1996; Flaspohler et al. 2010). Understanding the effects of forest fragmentation on biodiversity is essential for successful and efficient forest conservation (Estreguil and Mouton 2009) but this topic at present remains ambiguous. In particular, according to the island biogeography theory (MacArthur and Wilson 1967), forest fragmentation can be expected to cause local extinctions of native forest species, and fragmented forests will contain fewer of the original species of the forest than non-fragmented ones (Zuidema et al. 1996). Conversely, Forman (1995) postulated a general principle whereby larger patches, which are more environmentally heterogeneous, host greater species richness than do smaller patches. Recently Rosati et al. (2010) illustrated this theory by using an example of Mediterranean forests where a positive correlation between patch size and diversity value of vascular plants was found. Recent research has established

that some forest species are more sensitive to fragmentation than others (for a review see Liernet 2004). For instance, abundant characteristic species, the presence and fluctuations of which reflect the presence and fluctuations of other species in the community, can be of overriding importance for an ecosystem. Studying habitat fragmentation effects on abundant plant species that form the matrix of the community is of high scientific and conservation interest (Liernet 2004). In nature conservation and management, the identification of these species is of great value for the drafting and application of specific environmental legislation. In fact, EC Directive 92/43/EEC (Habitats Directive) (EEC 1992), one of the major steps toward a European strategy for nature conservation, lists a series of diagnostic species for habitats of conservation interest. For example, diagnostic species indicated in the Directive for forest habitats play a major role in determining the structure and functioning of these systems as, directly or indirectly, they control the availability of resources for other species. Moreover, these species can cause significant changes to their environment allowing the creation, modification or maintenance of the surrounding habitat. Although the ecological role of diagnostic species has been recognised (Chytrý et al. 2002), recent literature has only documented the responses of individual plant species and forest types in Europe to certain specific landscape threats (e.g. Honnay et al. 1999a,b; Kolb and

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Diekmann 2004, 2005; Rosati et al. 2010). The sensitivity of the assemblage of diagnostic species (sensu 92/43/EEC Directive Habitats) (EEC 1992) to forest fragmentation still requires exploration.

It has also been documented that fragmentation creates edge habitats (Forman and Godron 1986) that are characterised by different environmental conditions compared with interior forest habitats (Forman and Moore 1992). Finally, a small number of studies have underlined the importance of auto-ecological data for describing the relationship between the dimensions of habitat fragments and the spatial distribution of the species occupying them (Soulé and Simberloff 1986; Zimmerman and Bierregaard 1986). Analysis of the ecological and structural features of plant species (e.g. life forms, Ellenberg indicator values) has been broadly used to (a) compare plant communities (e.g. Diekmann et al. 1999; Chiarucci and Bonini 2005; Feola et al. 2011); (b) indirectly assess environmental conditions (e.g. Diekmann 1994; Falkengren-Grerup and Schöttelndreier 2004; Härdtle et al. 2005); and (c) describe the environmental settings of natural succession (e.g. Persson 1980; Dzwonko and Loster 1997; Dzwonko 2001). However, integrating investigations of the above-mentioned structural and ecological features with biodiversity analysis to better understand forest fragmentation is still required.

In consideration of the factors noted above, the present work analysed the structural, ecological and richness patterns of all native species versus diagnostic species only (based on Habitats Directive 92/43/EEC) (EEC 1992) in fragmented *Fagus sylvatica* forests of central Italy. In particular, we contrasted beech forests with different levels of fragmentation, focusing on floristic information, structural parameters (life forms), standard abiotic ecological

features (Ellenberg's indicator values) and the distribution of edge and clearing species. We investigated beech forests for two primary reasons. First, beech forests are widespread throughout Europe (Figure 1) and are currently distributed in a mosaic of patches with different fragmentation levels, which derive from centuries of cutting and livestock grazing (Diekmann et al. 1999), followed by a recent abandonment of cultural practices and a subsequent spontaneous reforestation process (Carranza et al. 2003; Van Gils et al. 2008). Second, beech forest ecosystems are a high conservation priority in Europe because they contain many species with poor dispersal (Diekmann et al. 1999). Consequently, the majority of European beech forests have been included in the European Habitats Directive (92/43/EEC) (EEC 1992).

The aim of this study was to compare the diversity patterns of diagnostic species assemblages (sensu 92/43/EEC) with the diversity patterns of all native species in fragmented beech forests with the intention of verifying if the assemblage of diagnostic species were useful indicators of forest fragmentation. Our expectation was that the set of diagnostic species, being strictly adapted to a particular forest habitat (in this case beech forests), could be more sensitive to forest fragmentation than the overall pool of native species. This would lead to marked differences in diversity patterns between those abundant characteristic species and the native species pool as a whole in more fragmented forests. If significant differences in life forms and ecological indicator values can be documented to show that diagnostic species are particularly sensitive to forest fragmentation, a consistent decline in diagnostic species diversity could be used as a sign of loss of diversity and ecological function. Therefore, understanding the composition,

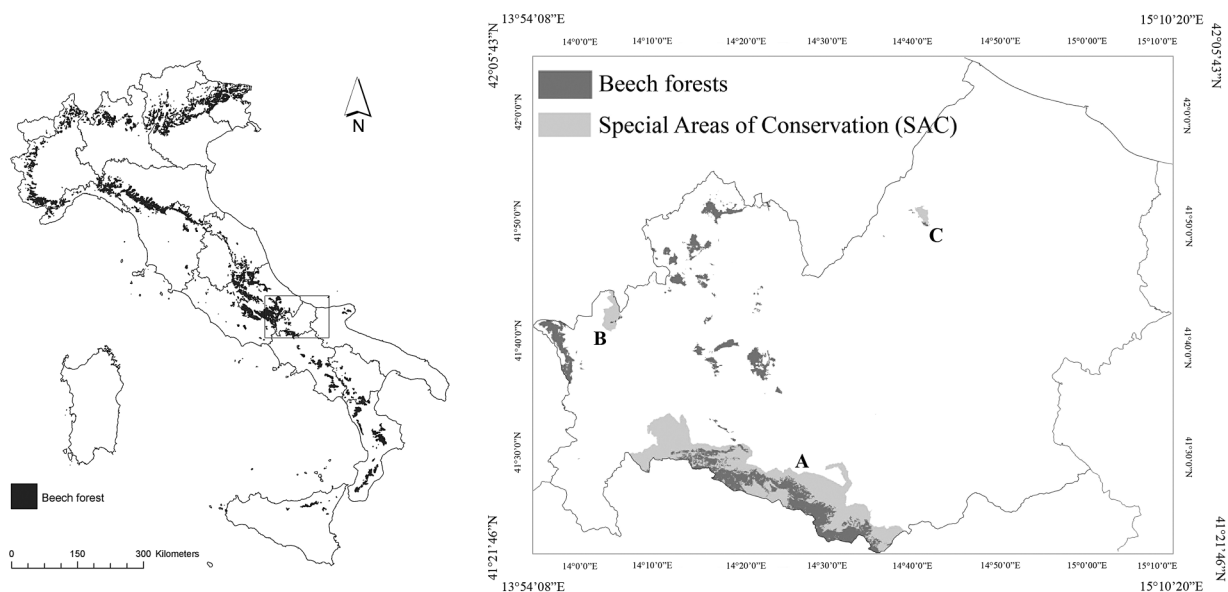


Figure 1. Location of the study area in the Italian distribution range of *Fagus sylvatica* (from CORINE Land Cover 4th level, <http://www.clc2000.sinanet.apat.it/>). On the left, the distribution map of *Fagus sylvatica* forests in the Molise Region according to Garfi et al. (2011) and location of SACs (<http://natura2000.eea.europa.eu/#>). A) IT7222287: La Gallinola-Monte Miletto-M. del Matese; B) IT7212126: Pantano della Zittola-Feudo Valcocchiara; C) IT7222211: Monte Mauro-Selva di Monte Falcone. The coordinate system is in UTM WGS84 33T.

structure and dynamics of beech forest fragments could help to better orient specific conservation actions and forest management strategies for beech forests.

Methods

Study area

The present study was conducted in *Fagus sylvatica* forests (“Apennine beech forests with *Taxus* and *Ilex*”– habitat of community interest, code 9210* in 92/43/EEC) included in three Special Areas of Conservation (SAC) (sensu Habitats Directive) (EEC 1992) located in the central Apennines, Molise Region, Italy (Figure 1). These forests are characterised by a main tree layer dominated by *Fagus sylvatica* (van Gils et al. 2008) and an herbaceous layer composed of sciophilous-nemoral species (Pignatti 1998).

Data collection

Plant species data were collected following a random stratified sampling design. According to the Regional Forest Management Plan (Anon 2002) and historic aerial photographs the analysed areas, were covered by woody vegetation during the last 60 years. The random sampling was performed based on a detailed (1:10,000 scale) and accurate ($\kappa = 0.81$) beech forest map (Garfi et al. 2011) that was previously classified into three fragmentation levels (for details see Frate et al. 2011), based on five patch metrics (McGarigal and Marks 1995) widely used to describe forest fragmentation (Haines-Young and Chopping 1996) (patch area in hectares, PA; perimeter in meters, P; shape index, SI; core area in hectares, Co; and Euclidean nearest neighbour in meters, ENN). Frate et al. (2011) classified beech forest patches according to their spatial pattern metrics by using a multivariate analysis procedure and identified the following three levels of fragmentation: highly fragmented, medium fragmented and non-fragmented (Table 1). The highly fragmented (H-Fr) class is characterised by small (PA = 1.55–74.05 ha), regularly shaped (SI = 1.36 and 64; P = 680–9140 m) and isolated patches (ENN = 40.84–519.81 m) with modestly sized core areas (Co = 0.74–30.75 ha). The medium fragmented (M-Fr) class contains medium-sized patches (PA = 1005–1721 ha) that are relatively closely spaced (ENN = 39.46 m) with medium-sized core areas (Co = 781–1540 ha) that are quite irregularly shaped

Table 1. Fragmentation classes of 37 beech forest stands in central Italy and their respective mean spatial pattern metrics. H-Fr, highly fragmented; M-Fr, medium fragmented; N-Fr, non-fragmented; N, number of stand studied; PA, Patch area in ha; P, Perimeter in m; SI, Shape Index; Co, Core Area in ha; ENN, Euclidean Nearest Neighbour in m (From Frate et al. 2011).

	N	PA	P	SI	Co	ENN
H-Fr	10	23	3260	1.83	18.29	259.41
M-Fr	12	1364	149290	10.52	1160.73	23.46
N-FR	15	4249	284740	10.92	3865.6	7.46

(SI = 8.05–12.98 and P = 133,740–164,840 m). Finally, the non-fragmented (N-Fr) class contains very large (PA > 4300 ha; Co > 3860 ha), irregularly shaped (SI = 10.92 and P = 284,770 m) and connected patches (ENN < 50 m). For collecting plant species data, a 50 m regular grid was placed over the beech forest map in a Geographic Information System (GIS) environment. Using this grid, the sample areas were randomly selected within each fragmentation level, excluding border cells (those with a forest cover of less than 80%). Selected grid cells were later identified in the field with the help of the beech forest map, topographic maps (scale 1:5,000) and high-resolution orthorectified aerial photographs (1 pixel = 0.25 m²). The number of sampling units was proportional to the cover of each fragmentation class and resulted in a distribution as follows: 10 sample areas in highly fragmented forests, 12 in medium-fragmented forests and 15 in non-fragmented forests. In the centre of each sample grid cell, a 10 m × 10 m plot was laid out and divided into four equal quadrants (5 m × 5 m). For each plot, a complete list of vascular plant species was compiled and a frequency value (ranging from 1–4) was assigned to each species in accordance with the number of plot quadrants in which it was present. The nomenclature of sampled species conformed to the checklist of Italian vascular flora (Conti et al. 2005). Diagnostic species for the ‘Apennine beech forests with *Taxus* and *Ilex*’ (code 9210*) habitat type were identified based on the ‘Interpretation Manual of European Union Habitats’ (European Commission 2007) and the ‘Italian Interpretation Manual of the 92/43/EEC Directive habitats’ (Biondi et al. 2009).

Data analysis

Describing the diversity patterns of vascular plants in fragmented and non-fragmented patches. To estimate the overall species richness at the three levels of fragmentation, plot-based rarefaction curves were calculated (Gotelli and Colwell 2001), using an analytical formula (Chiarucci et al. 2008b) and the software EstimateS (version 7.5) (Colwell 2004). To compare species richness among the levels of fragmentation at a standardised number of plots (\hat{S}_{MaoTao}), 95% confidence intervals were also assessed. The same procedure was applied for the diagnostic species pool. The ratio between the rarefaction curves of diagnostic species and all species was also calculated (Chiarucci et al. 2008a; Stanisci et al. 2010; Santoro et al. 2012). This ratio was utilised to test whether diagnostic species represented a constant proportion of all species or followed some specific accumulation pattern.

Identifying the ecological and structural characteristics of vascular plant species in fragmented and non-fragmented patches. The structural, ecological and functional characteristics of each level of fragmentation were examined, using life forms (according to Pignatti 1982), Ellenberg ecological indicator values (Ellenberg 1974; Pignatti et al. 2005) and the distribution of clearing and edge species

(Pignatti 1982). The Ellenberg indicator values considered were light (L), temperature (T), moisture (M), soil reaction (R) and soil nitrogen (N). Based on Pignatti et al. (2005), the continentality value (C) was not considered because it is insignificant for Italian vegetation. These indicator values are based on the assumption that most plants may serve as bioindicators (Diekmann 2003), i.e. they can provide information on the ecological features of the environment. For each plant species, Ellenberg indicator values are expressed as a range of values from 1–9 or from 1–12. For comparing the ecological indicator values of the sampled flora in the different strata (fragmentation levels), we used weighted average values (WA) because they are reliable predictors of site conditions (Diekmann 1995). WA values were calculated using quantitative data (frequency). The sampled plant species were also classified into functional classes according to the species ecology described by Pignatti (1982). In particular, we distinguished clearing species from non-clearing species and edge species from non-edge and core species.

For each ecological, structural and functional parameter, we carried out statistical analyses to quantify the differences between the three levels of fragmentation. We assessed the significance of the differences between groups using the non-parametric Kruskal–Wallis test. Finally, the behaviour of each ecological and structural parameter in the three fragmentation classes was graphically compared using box plots.

Results

Species richness and diversity

The recorded species list consisted of 158 taxa that represented ca. 80% of the vascular plant species of beech forests in central Italy (Abbate et al. 2003). We identify 33 diagnostic species which represented ca. 21% of the sampled flora. Several species, such as *Aremonia agrimonoides*,

Cardamine bulbifera, *Fagus sylvatica*, *Galium odoratum*, *Mycelis muralis*, *Rubus hirtus*, *Sanicula europaea* and *Viola reichenbachiana* were present at all three levels of fragmentation, while others, such as *Cardamine kitaibelii*, *Paris quadrifolia* and *Potentilla micrantha* were exclusive to non-fragmented forests (Table 2). Floristic analysis also identified a group of diagnostic species that were preferentially distributed in non-fragmented forests (e.g. *Actaea spicata*, *Cardamine kitaibelii*, *Doronicum orientale*, *Lathyrus venetus*, *Oxalis acetosella*, *Paris quadrifolia*, *Polystichum aculeatum* and *Taxus baccata*) (Table 1).

The distribution across the different levels of fragmentation of *Taxus baccata* and *Ilex aquifolium*, which are two relics of ancient Tertiary forests, followed two opposite trends. While *Taxus baccata* was present in less-fragmented forests, *Ilex aquifolium* was preferentially present in more-fragmented forests. Rarefaction curves of the entire pool of species showed a similar pattern for highly and medium-fragmented patches, and always resulted in values above those of the non-fragmented woodlands. In contrast, rarefaction curves that considered only the diagnostic species exhibited the opposite pattern. In fact, the rarefaction curve of diagnostic species for non-fragmented beech forests was above those with medium and high fragmentation values (Figure 2).

The curve derived from the ratio of diagnostic to all vascular species (Figure 2) showed differences in the spatial distribution of the two groups across the three levels of fragmentation. The ratio curve for highly fragmented forests was almost flat, indicating that diagnostic species had the same accumulation pattern as the entire pool of species; therefore, their contribution to diversity was constant. In contrast, the ratio curve for non-fragmented forests was steeply descending, denoting that diagnostic species were notably common and that a few plots were sufficient to sample the majority of these taxa.

Table 2. Distribution of diagnostic species (sensu 92/43/EEC directive) in the three levels of fragmentation. N-Fr, non-fragmented; M-Fr, medium fragmentation; H-Fr, high fragmentation. Values are expressed as percent frequency recorded in the quadrants sampled (numbers in parentheses).

Species	N-Fr (60)	M-Fr (48)	H-Fr (40)	Species	N-Fr (60)	M-Fr (48)	H-Fr (40)
<i>Abies alba</i>	13.3	0.0	5.0	<i>Galanthus nivalis</i>	0.0	2.0	5.0
<i>Acer platanoides</i>	0.0	2.1	0.0	<i>Galium odoratum</i>	55.0	54.1	12.5
<i>Acer pseudoplatanus</i>	3.3	2.1	7.5	<i>Ilex aquifolium</i>	1.7	14.6	17.5
<i>Actaea spicata</i>	5.0	4.2	0.0	<i>Lathyrus vernus</i>	16.7	16.7	7.5
<i>Anemone apennina</i>	20.0	22.9	20.0	<i>Melica uniflora</i>	11.7	14.6	32.5
<i>Anemone nemorosa</i>	5.0	0.0	17.0	<i>Mycelis muralis</i>	40.0	41.7	27.5
<i>Aremonia agrimonoides</i>	46.7	52.1	27.5	<i>Oxalis acetosella</i>	23.3	8.3	0.0
<i>Cardamine bulbifera</i>	70.0	39.6	67.5	<i>Paris quadrifolia</i>	3.3	0.0	0.0
<i>Cardamine chelidonia</i>	1.7	2.1	2.5	<i>Polygonatum multiflorum</i>	10.0	14.6	15.0
<i>Cardamine kitaibelii</i>	30.0	0.0	0.0	<i>Polystichum aculeatum</i>	6.7	8.3	0.0
<i>Cephalanthera damasonium</i>	18.3	47.9	20.0	<i>Potentilla micrantha</i>	1.7	0.0	0.0
<i>Corydalis cava</i>	6.7	0.0	5.0	<i>Ranunculus lanuginosus</i>	40.0	10.4	20.0
<i>Doronicum orientale</i>	6.7	10.4	0.0	<i>Rubus hirtus</i>	56.7	68.8	75.0
<i>Dryopteris filix-mas</i>	6.7	10.4	12.5	<i>Sanicula europaea</i>	58.3	33.3	20.0
<i>Euphorbia amygdaloides</i>	23.3	10.4	17.5	<i>Taxus baccata</i>	5.0	2.1	0.0
<i>Fagus sylvatica</i>	91.7	95.8	87.5	<i>Viola reichenbachiana</i>	86.7	70.8	55.0

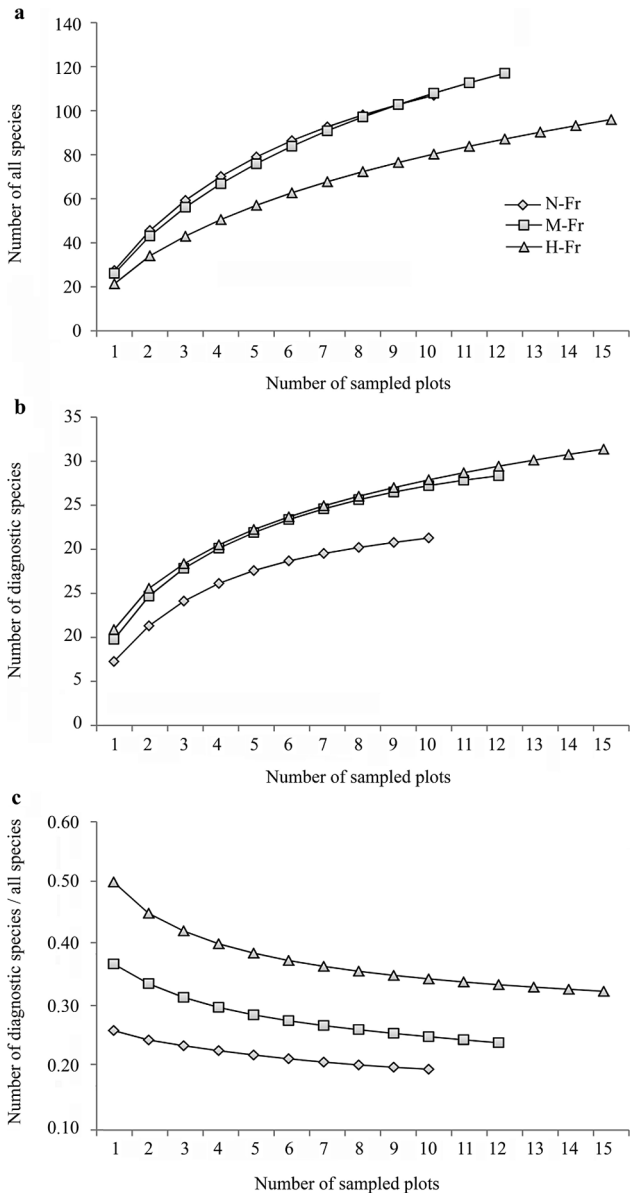


Figure 2. Plot-based rarefaction curves for (a) all recorded species, (b) diagnostic species, and (c) the ratio between the rarefaction curves of diagnostic and all species in non-fragmented (N-Fr), medium fragmented (M-Fr), and highly fragmented forest stands (H-Fr).

Life forms

Consistent differences were evident in the distribution of life forms among the three levels of fragmentation (Figure 3). Therophytes were abundant in fragmented forests and significantly less abundant in non-fragmented forests compared with both medium fragmented (Kruskal–Wallis test, $P < 0.01$; d.f. = 2) and highly fragmented forests (Kruskal–Wallis test, $P < 0.05$). The correlation between the abundance of phanerophytes and the degree of fragmentation was also positive, and was significantly different between non-fragmented and highly fragmented forests (Kruskal–Wallis test, $P < 0.05$), as well as between medium and highly fragmented forests (Kruskal–Wallis test, $P < 0.01$). In addition, the abundance of geophytes

tended to decrease in fragmented patches, but no significant differences were evident. No other significant structural differences resulted of the analysis because chamaephytes and hemicryptophytes did not follow any trend.

Ellenberg's indicator values

Significant ecological differences emerged between fragmentation levels when the Ellenberg light indicator was studied (Figure 4). The light index increased with the degree of fragmentation, and there was a significant difference between non-fragmented and medium fragmented forests (Kruskal–Wallis test, $P < 0.05$), as well as between non-fragmented and highly fragmented forests (Kruskal–Wallis test, $P < 0.01$). However, no significant differences between fragmentation levels were evident for moisture, temperature, soil reaction or nitrogen (Figure 4).

Presence of clearing and edge species

A total of 15 clearing species and 18 edge species (sensu Pignatti 1982) were recorded in the three levels of fragmentation. Their mean richness per plot was positively correlated with the degree of fragmentation (Figure 5), with significant differences between non-fragmented and highly fragmented forests for clearing species (Kruskal–Wallis test, $P = 0.05$). There were also significant differences between non-fragmented and medium-fragmented (Kruskal–Wallis test, $P < 0.01$), as well as between non-fragmented and highly fragmented forests (Kruskal–Wallis test, $P < 0.05$) for edge species. In particular, a group of nine clearing species (*Agrostis capillaris*, *Aristolochia rotunda*, *Cardamine graeca*, *Dactylis glomerata*, *Festuca circummediterranea*, *Poa bulbosa*, *Satureja hortensis*, *Senecio squalidus* and *Trifolium pratense*) and nine edge species (*Campanula persicifolia*, *Crataegus laevigata*, *Dactylorhiza maculata*, *Lilium bulbiferum*, *Loncomelos pyrenaicus*, *Ostrya carpinifolia*, *Sesleria autumnalis*, *Silene italica* and *Silene nutans*) were absent in non-fragmented forests and increased at the medium and highly fragmented sites (Table 3).

Discussion

We found a consistent relationship between overall species diversity and fragmentation level; the species richness was lower in non-fragmented forests and higher in very fragmented ones. A similar distribution pattern for species richness has already been described for a relict deciduous forest in Belgium, where smaller fragments contained almost the entire pool of species of the region (Honnay et al. 1999b). In our case, the high plant species richness in fragmented patches represents the influence on forest diversity of both the forest edges and the neighbouring habitats. Floristic analysis shows, as previously described for other European temperate forest types (for a review see Liernet 2004), the presence of a consistent cohort of edge and clearing species in fragmented beech

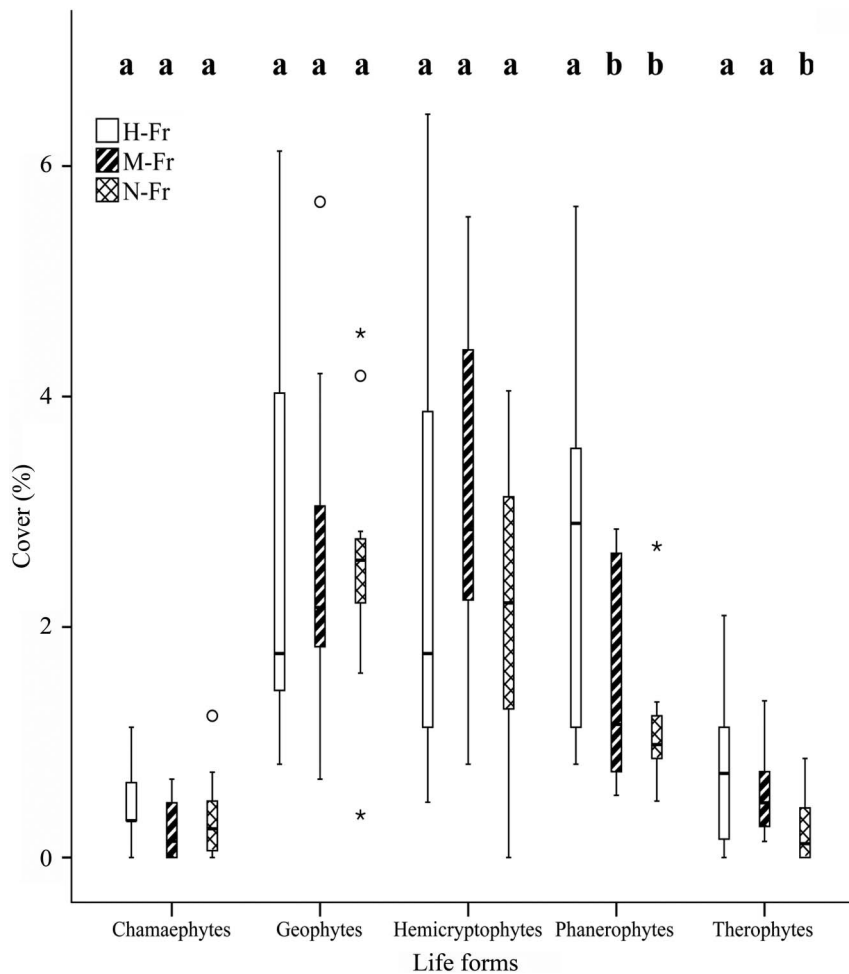


Figure 3. Box and whisker plots of life form frequencies in non-fragmented (N-Fr), medium fragmented (M-Fr), and highly fragmented (H-Fr) beech forest stands in central Italy. Letters represent homogenous subsets according to Kruskal–Wallis test at $\alpha = P < 0.05$.

forests, many of them exclusively present in highly fragmented forests (Table 3). Note that the highly fragmented forests investigated in this study are represented by small patches that rarely exceeded 10 ha, and the diversity and ecology of such small patches is generally strongly influenced by edge habitats (Kapos 1989; Zuidema et al. 1996; Viana et al. 1997). In addition, many species of contiguous woody communities characterised by a more open canopy structure and by higher plant diversity, such as oak forests and other hardwood forests (Pignatti 1998), colonise more-fragmented beech forests (e.g. *Cornus mas*, *Cornus sanguinea*, *Euonymus europaeus*, *Fraxinus ornus*, *Ligustrum vulgare* and *Lonicera caprifolium*). In other words, fragmented beech forests exhibit higher diversity values than non-fragmented forests, due to both the increase in forest edges that host a high number of species (Fahrig 2003) and the influx of species from neighbouring habitats (e.g. scrub, grassland).

In contrast with previous research on temperate woodlands (Piessen et al. 2005; Rosati et al. 2010), non-fragmented Apennine beech forests exhibit low values of plant species richness relative to more-fragmented forests.

For instance, Rosati et al. (2010) found high values of diversity in large oak deciduous forests patches (mean extension of 285 ha) and established that such biodiversity peaks respond to the fine-scale mosaic of microhabitats inside them. Instead, our findings are consistent with previous monographic research (Pignatti 1998) that described the mature beech forests in central Italy as notably homogeneous communities characterised by lower diversity values compared with other neighbouring communities, such as oak forests and other hardwood forests, which are characterised by a more open and heterogeneous canopy structure. Among the natural causes of such reduced plant diversity, Pignatti (1998) mentioned the competitiveness and resistance to cold stress of *Fagus sylvatica* relative to other tree species. In accordance with Piovesan et al. (2009), the distribution of *Taxus baccata* was mainly confined to remote localities or to areas where cultural practices were absent. Conversely, *Ilex aquifolium*, which characterises mature forests, but can colonise other deciduous woods (Pignatti 1998), while preferentially distributed in non-fragmented forests, was also present in medium and highly fragmented forests.

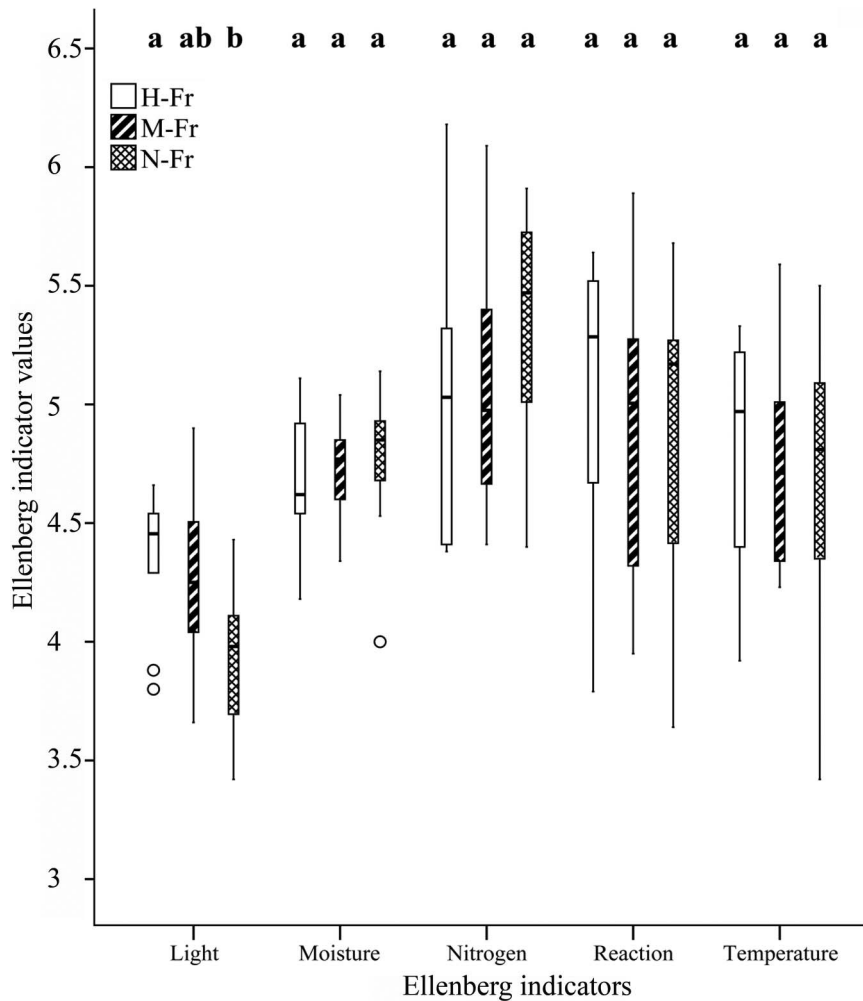


Figure 4. Box and whisker plots of Ellenberg indicator values in non-fragmented (N-Fr), medium fragmented (M-Fr), and highly fragmented (H-Fr) beech forest stands in central Italy. Letters represent homogenous subsets according to Kruskal–Wallis test at $\alpha = P < 0.05$.

The analysis of structural data showed a significant increase in phanerophytes and therophytes across fragmentation levels of beech forests. A similar increase in phanerophytes in fragmented American beech forests has previously been described by Palik and Murphy (1990), who claimed that fragmented patches hosted a greater number of tree species because fragmentation increased light availability, thereby promoting the expansion of phanerophytes that originated from neighbouring forests or were typical of early succession stages. The increment of phanerophytes in fragmented woodlands is particularly striking in *Fagus sylvatica* forests, where the canopy structure in well-conserved formations is notably simple and homogeneous (Pignatti 1998; Piovesan et al. 2005). Furthermore, the structure of beech forests in central Italy, as characterised by the near-absence of annual herb species and by a poor cohort of woody species (Piovesan et al. 2005), was consistently altered in fragmented patches, due to the advent of species, such as therophytes and phanerophytes, typical of forests with a more open canopy.

Geophytes, which are typical of mature and well-structured forests (Pignatti 1998), tended to have a higher abundance in medium- and non-fragmented patches. We found a significant increase in the Ellenberg indicator light index in more-fragmented forests. Similar results were previously found by Lucchese and Monterosso (1994) in disturbed beech forests. Furthermore, Palik and Murphy (1990) noted that the distribution of herbaceous plant species in a forest was strongly dependent on light penetration, and that an increase in light in the lower layers of fragmented forests was usually marked by an increase in heliophilous species and a decrease in sciophilous species. Accordingly, we found a group of sciophilous species (absolute value of Ellenberg indicator for light compressed between 1 and 3; e.g. *Actaea spicata*, *Cardamine kitaibelii*, *Dryopteris affinis*, *Hepipactis helleborine*, *Oxalis acetosella*, *Paris quadrifolia*, *Polystichum aculeatum*, *Sesleria autumnalis* and *Taxus baccata*) that were exclusively present in medium and non-fragmented beech forests. In contrast, we identified heliophilous species (e.g. *Arabis alpina* and

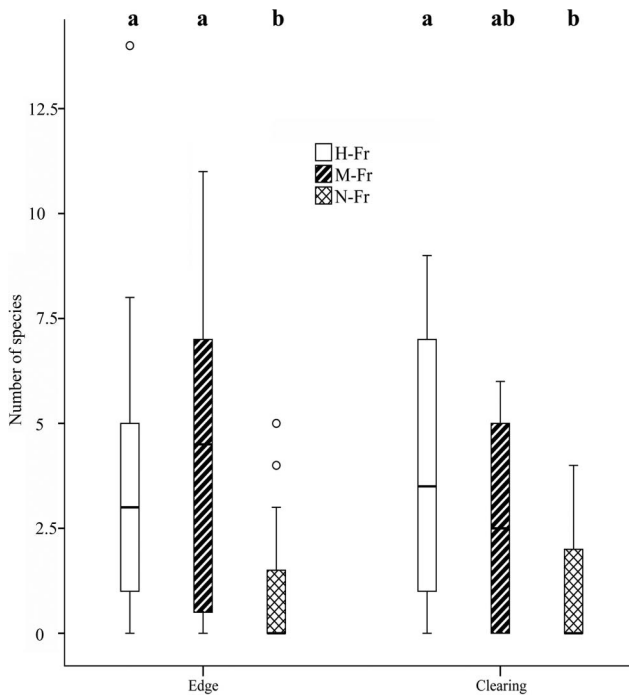


Figure 5. Box and whisker plots of clearing and edge species frequency in non-fragmented (N-Fr), medium fragmented (M-Fr), and highly fragmented (H-Fr) beech forest stands in central Italy. Letters represent homogenous subsets according to Kruskal–Wallis test at $\alpha = P < 0.05$.

Ceterach officinarum) that were present only in fragmented patches. The other Ellenberg indicators (temperature, moisture, soil reaction and nitrogen) presented similar values for the different fragmentation levels and confirmed the expected average values reported by Pignatti (1998) for Apennine beech woods.

Regarding the distribution of edge and clearing species (Pignatti 1982), as expected (e.g. Honnay et al. 1999a; Tabarelli et al. 1999; Liernet 2004; de Albuquerque and Rueda 2010), we observed an increase in generalist species, such as *Cardamine graeca* and *Galium aparine*, in fragmented forests and a simultaneous decrease in a number of true forest species (sensu Hermy et al. 1999), such as *Cardamine kitaibelii* and *Paris quadrifolia*.

The analysis of the species rarefaction curves for the EEC habitat diagnostic species alone showed higher values in non-fragmented beech forests than at the other levels of fragmentation. The highest richness of beech forest diagnostic species was evident in non-fragmented patches, a result confirmed by the ratio curve between diagnostic species and the entire pool of species. This ratio highlights that diagnostic species are widespread and well distributed in non-fragmented forests but are negatively affected by fragmentation. Our results indicate that these species form a robust group of forest specialist species (sensu Peterken and Game 1984; Dzwonko and Loster 1989; Dupré and Ehrlén 2002; Kolb and Diekmann 2005) that could be used for describing and monitoring fragmentation and its impacts. Furthermore, floristic analysis allowed us to identify the presence in non-fragmented forests of ‘ancient forest species’ (sensu Hermy et al. 1999) (e.g. *Actaea spicata*, *Oxalis acetosella*, *Paris quadrifolia* and *Polystichum aculeatum*), which suggest a long, continuous history for the habitat patch. Ancient species are those taxa that may take centuries to colonise a forest and are indicative of more typical forest conditions (Hermy et al. 1999). These species are important in terms of nature conservation because the list of ancient forest species combines both qualitative (forest quality) and quantitative (diversity) conservation criteria (Honnay et al. 1999a). Two species, *Cardamine kitaibelii* and *Paris quadrifolia*, were exclusively present

Table 3. Distribution of edge and clearing species at the three levels of fragmentation. N-Fr, non-fragmented; M-Fr, medium fragmentation; H-Fr, high fragmentation. Values are expressed as percent frequency recorded in the quadrants sampled (numbers in parentheses).

Edge species	N-Fr (60)	M-Fr (48)	H-Fr (40)	Clearing species	N-Fr (60)	M-Fr (48)	H-Fr (40)
<i>Arabis turrata</i>	1.7	12.5	10.0	<i>Agrostis capillaris</i>	0.0	12.5	15.0
<i>Arum italicum</i>	6.7	0.0	0.0	<i>Anthriscus caucalis</i>	1.7	0.0	0.0
<i>Campanula persicifolia</i>	0.0	4.8	0.0	<i>Aristolochia rotunda</i>	0.0	0.0	2.5
<i>Campanula trachelium</i>	1.7	25.0	27.5	<i>Campanula glomerata</i>	1.7	0.0	0.0
<i>Crataegus laevigata</i>	0.0	4.2	15.0	<i>Cardamine graeca</i>	0.0	8.3	30.0
<i>Dactylorhiza maculata</i>	0.0	2.1	2.5	<i>Cruciata laevipes</i>	1.7	0.0	0.0
<i>Dianthus barbatus</i>	1.7	0.0	0.0	<i>Dactylis glomerata</i>	0.0	2.1	0.0
<i>Digitalis lutea australis</i>	6.7	16.7	2.5	<i>Festuca circummediterranea</i>	0.0	2.1	0.0
<i>Helleborus foetidus</i>	1.7	2.1	7.5	<i>Galium aparine</i>	1.7	22.9	30.0
<i>Hieracium racemosum</i>	1.7	2.1	0.0	<i>Poa bulbosa</i>	0.0	2.1	0.0
<i>Lamium flexuosum</i>	3.3	4.2	7.5	<i>Satureja hortensis</i>	0.0	2.1	0.0
<i>Lilium bulbiferum</i>	0.0	8.3	12.5	<i>Senecio squalidus</i>	0.0	4.2	0.0
<i>Loncomelos pyrenaicus</i>	0.0	0.0	7.5	<i>Silene latifolia</i>	1.7	0.0	0.0
<i>Ostrya carpinifolia</i>	0.0	2.1	0.0	<i>Stellaria media</i>	16.7	6.3	15.0
<i>Sesleria autumnalis</i>	0.0	6.3	0.0	<i>Trifolium pratense</i>	0.0	2.1	0.0
<i>Silene italica</i>	0.0	16.7	2.5				
<i>Silene nutans</i>	0.0	0.0	5.0				
<i>Stellaria holostea</i>	1.7	0.0	0.0				

in non-fragmented forests. This exclusive distribution and the fact that Pignatti (1998) defined these two species as characteristic of well-structured beech forests in central Italy suggest we should consider them as indicators of non-fragmented beech forests.

The observed results and the controversial interpretation of the relationship between species richness and fragmentation (Ochoa-Gaona et al. 2004) suggest that overall richness is not an appropriate indicator for characterising forest fragmentation. Instead, our results established that the integration of floristic analysis with structural and ecological characterisation, paying particular attention to certain diagnostic groups, is more sensitive than species richness per se. Irrespective of cause–effect, our results indicate that patterns of overall native species richness and diagnostic species richness in relation to fragmentation may not be congruent. Diagnostic species can provide information that would be missed when considering all native species as a group. When considering the relationship between forest fragmentation and the richness patterns of native species, conservation managers should be aware that even if no significant differences in overall native richness are observed, changes in specific assemblages could exist (Chiarucci et al. 2008a). Moreover, if these changes correspond to diagnostic species, which are conservation targets as well as important indicators of habitat conservation status, the importance of such observed changes should not be underestimated. In certain cases, a consistent decline in diagnostic species diversity could be used as a signal of losses in diversity, structure and ecological functions. Accordingly, the diversity patterns of diagnostic species could help to define specific conservation actions to prevent a decrease in overall species diversity.

In this context, the analysis of diversity patterns focusing on an appropriate species pool represents an instrument for assessing and monitoring fragmentation processes in other forest ecosystems. Understanding the ecological impacts of fragmentation is an essential first step in determining conservation strategies for fragmented forests. Therefore, the distinction between total species richness and the presence of characteristic (diagnostic) species in native species assemblages and their integrated analysis with structural parameters (life forms) and standard ecological features (Ellenberg's indicator values), as proposed in our study, could be a useful tool for forest management and should be considered in the planning of restoration projects.

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CAPITOLO 7

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Regional-scale modelling of the cumulative impact of wind farms on bats

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Abstract Wind farms are steadily growing across Europe, with potentially detrimental effects on wildlife. Indeed, cumulative impacts in addition to local effects should be considered when planning wind farm development at a regional scale, and mapping the potential risk to bats at this scale would help in the large-scale planning of wind turbines and focus field surveys on vulnerable areas. Although modelling offers a powerful approach to tackle this goal, its application has been thus far neglected. We developed a simple regional-scale analysis in an area of central Italy (Molise region) that is undergoing considerable wind farm development. We implemented species distribution models (SDMs) for two bat species vulnerable to wind farm impact, *Nyctalus leisleri* and *Pipistrellus pipistrellus*. We developed risk maps by overlaying SDMs for the two species with turbine locations, assessed the alteration of the landscape patterns of foraging habitat patches determined by the wind turbines, and identified highly vulnerable areas where wind farm construction would be particularly risky. SDMs were statistically robust ($AUC \geq 0.8$ for both species) and revealed that 41 % of the region offers suitable foraging habitat for both species. These areas host over 50 % of the existing or planned wind farms, with 21 % of the turbines located within 150 m of forest edges, suggesting an increase in fatality risk. The alterations in suitable foraging patches consisted of a 7.7 % increase in the number of patches, a 10.7 % increase in the shape index, and a 8.1 % decrease in the

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mean patch area. The region's western portion, which is most suitable to both species, requires careful consideration with regard to future wind farm planning.

Keywords Chiroptera · Energy · Habitat alteration · Maxent · Risk assessment · Species distribution models

Abbreviations

AI	Aggregation index
AUC	Area under the receiver operating characteristic curve
AWMSI	Area weighted mean shape index
CA	Class area
CLC	Corine land cover
FFT	Fast fourier transformation
LPA	Landscape pattern analyses
LPI	Largest patch index
MPS	Mean patch size
NP	Number of patches
SDM	Species distribution models

Introduction

The wind farm industry is the most important source of renewable energy in Europe (Harbusch and Bach 2005). Although wind energy represents a valuable alternative to mitigate the detrimental effects of carbon emission-related global warming on wildlife (Arnett 2005), wind farms have been found to affect wildlife, particularly flying vertebrates, indirectly through habitat loss and directly by increasing their mortality rate due to direct collisions with the turbines (Johnson and Erickson 2003; Telleria 2009).

Evaluating the magnitude of risk prior to wind farm construction appears to be the most promising strategy to identify sites at which the potential impact of wind turbines on wildlife would be minimized (Larsen and Madsen 2000). For several years, impact evaluations on wildlife have mostly targeted birds (Rodrigues et al. 2008). However, attention to bats has increased after the publication of two papers by Rahmel et al. (1999) and Bach et al. (1999). Furthermore, recent studies have described the collision impacts on these mammals at a local scale (Johnson et al. 2000; Johnson and Erickson 2003; Hötter et al. 2006; Telleria 2009). In Europe, the agreement on the conservation of populations of European bats (EUROBATS) has issued specific guidelines (Rodrigues et al. 2008), setting standards for the appropriate consideration of bats in wind farm planning and describing methods for bat surveys prior to construction and monitoring once the wind turbines are operating. Although bats are well known to be sensitive to wind farms (Jaberg and Guisan 2001; Arnett 2005; Rodrigues et al. 2008; Telleria 2009; Kunz et al. 2007b), there is little knowledge of the relative importance of different types of impacts, i.e., collision risk, habitat loss, and habitat fragmentation, particularly at a large geographical scale. Indeed, previous studies have focused on local assessments of risk, as based on survey data. However, because of the massive expansion of the wind farm industry throughout much of Europe, impact assessment should also carefully consider the cumulative regional impacts

of wind farms on sensitive wildlife (Jaberg and Guisan 2001; Rodrigues et al. 2008). One of the expected products of large-scale impact assessments is also the generation of maps of high-risk areas, which could provide a crucial tool to locate sites where wind farms cannot be planned or sites where more survey efforts are needed to assess the actual likelihood of impact (e.g., Cathrine and Spray 2009). Nevertheless, despite its importance, the assessment of cumulative impacts has been neglected to date. Species distribution models (SDMs) represent a potentially powerful approach to achieve this goal, though it has predominantly been applied to address other conservation issues (Ficetola et al. 2007; Elith et al. 2006; Heikkinen et al. 2007; Rebelo et al. 2009; Zimmermann et al. 2010; Rebelo and Jones 2010; Bosso et al. 2013). Thus far, SDMs have been applied to predict mortality risks for bats only in a single case study, covering all of Portugal (Santos et al. 2013); however, that model relied on the precise knowledge of bat mortality based on previous fatality reports. In many countries, such as Italy, data on bat mortality at existing wind farms are practically non-existent (Ferri et al. 2011) because the monitoring of such sites is not obligatory; therefore, generating risk maps using that approach is unfeasible. In such cases, it is crucial to follow a pragmatic approach to assess the cumulative impact of regional wind farm planning and to locate critical areas in the absence of mortality data. An alternative approach to a mortality-based model may be given by assessing the extent to which turbines are within areas of high habitat suitability for bats. As a first example to address this issue, we developed a regional-scale analysis based on SDMs and Landscape pattern analyses (LPAs) in an area in central Italy, the Molise region, which is currently undergoing large-scale development for wind farms. When land use is profoundly altered by the presence of wind turbines, e.g., when the farm requires a forest patch to be cleared (in countries where this is allowed), the bat species associated with the missing habitat type will be directly affected by its loss (Johnson and Erickson 2003). Furthermore, in the case of the loss of forest, both the species foraging in this habitat and those roosting in the trees will be markedly affected. However, habitat alteration may still occur when seemingly less dramatic changes are instituted. Even if prey availability remains unchanged, we assume that the presence of wind turbines in a foraging site could affect the quality of that site to a varying extent by altering the spatial structure and by forcing—or, according to some studies, even attracting—bats to forage near wind turbines, increasing mortality risk and turning the site into an ecological trap (Battin 2004; Rodrigues et al. 2008; Weller and Baldwin 2011; Rydell et al. 2012). In cases in which wind farm development leads to forest fragmentation, although fragmentation might, in principle, favour species adapted to forage in an edge habitat, the increased bat activity might expose bats to higher risks of collision with the nearby wind turbines. We used SDMs to identify areas characterized by a high habitat suitability for bats and located where these sites overlap with wind turbines. Although the impact of wind turbines on bats may be variable and site specific, our main goal is to offer a region-scale planning tool which would not replace, but only assist, pre-construction bat surveys. Therefore, we adopted a precautionary approach and assumed that the overlap between suitable habitat and wind turbines poses an additional risk to bats either by reducing suitable foraging and/or roosting habitat or, in cases in which habitat suitability is unaltered or even improved, by increasing collision likelihood, as explained above.

Additionally, wind farm development may increase fragmentation, even if it does not directly cause a reduction in connectivity: the matrix “hostility”, in this case, is represented by a potentially higher risk of mortality outside the unaltered habitat patch generated by the surrounding occurrence of wind turbines. A novel aspect of our model is that it incorporates the landscape pattern alterations determined by wind farms, which

potentially represents an important component of cumulative impact. We deliberately selected a regional rather than a national scale, as this is the geographical (and administrative) dimension at which wind farm development is planned and mitigation or compensation actions occur. Because all bat species occurring in Italy are included in Annex IV of Habitat Directive 92/43/EEC and 13 of them are featured in Annex II, the evaluation of the cumulative impact also represents a tool to pursue the objectives set by the Natura 2000 program in Europe (EEC 1992). We specifically built SDMs for two bat species already reported in the literature to be highly sensitive to wind farms: Leisler's bat *Nyctalus leisleri* and the common pipistrelle *Pipistrellus pipistrellus*. *N. leisleri* is a migrant species that usually flies at high altitudes (Waters et al. 1999) and is regarded as one of the most important bat species to be investigated in areas acutely affected by wind farms construction (Rodrigues et al. 2008). *P. pipistrellus* is known to be highly vulnerable to wind farms, particularly in the southern part of Europe and when foraging in areas where wind farms occur (Rodrigues et al. 2008; Telleria 2009). Such species are also good models because they are representative of different guilds, i.e., open space (*N. leisleri*) and edge (*P. pipistrellus*) foragers (Dietz et al. 2007). We combined species presence data with a set of environmental variables best matching the ecological needs of the species in terms of their foraging habitat requirements (Anderson and Martínez-Meyer 2004). The specific objectives of our analysis were as follows: (a) to develop risk maps by overlaying the foraging habitat maps obtained for the two species with the existing and planned wind farms locations; (b) to assess changes in the spatial pattern of foraging habitat determined by the existing and planned wind turbines; and (c) to identify highly vulnerable areas where wind farm construction would be particularly harmful to bats and should be avoided.

Materials and methods

Study area

The analyses were performed in the Molise region (central Italy), an area of 443,758 ha, which was selected as a model because it is one of the Italian areas undergoing a rapid increase in the number of wind turbines. Overall, 256 wind turbines are already operating in 28 wind farms, and 287 turbines are planned in 11 future wind farms, for a grand total of 39 wind farms and 543 turbines (1.22 turbines/ha) (Fig. 1).

Presence data

To implement SDMs, we used presence data collected in 58 sampling locations. The data were systematically gathered in 2010 at existing and planned wind farm locations and in control areas (Fig. 1). The data were obtained by recording echolocation and social calls with a bat detector in the time expansion mode or by direct ultrasound sampling (D240X and D1000X Pettersson bat detectors, respectively, Pettersson Elektronik AB, Uppsala, Sweden). The occurrence of feeding buzzes was regarded as evidence of bat foraging (e.g. Davy et al. 2007). We recorded bat occurrence with bat detectors at point locations for approx. 60 min/site starting at 30 min after sunset, i.e., when all bat species occurring in Italy are active; the sampling was as uniform as possible. As both species broadcast intense echolocation calls, they can be easily recorded with bat detectors. Overall, we identified 36 and 58 foraging areas for *N. leisleri* and *P. pipistrellus*, respectively. The sampling effort we adopted is appropriate to offer statistical support to the MaxEnt modelling environment (Phillips et al.

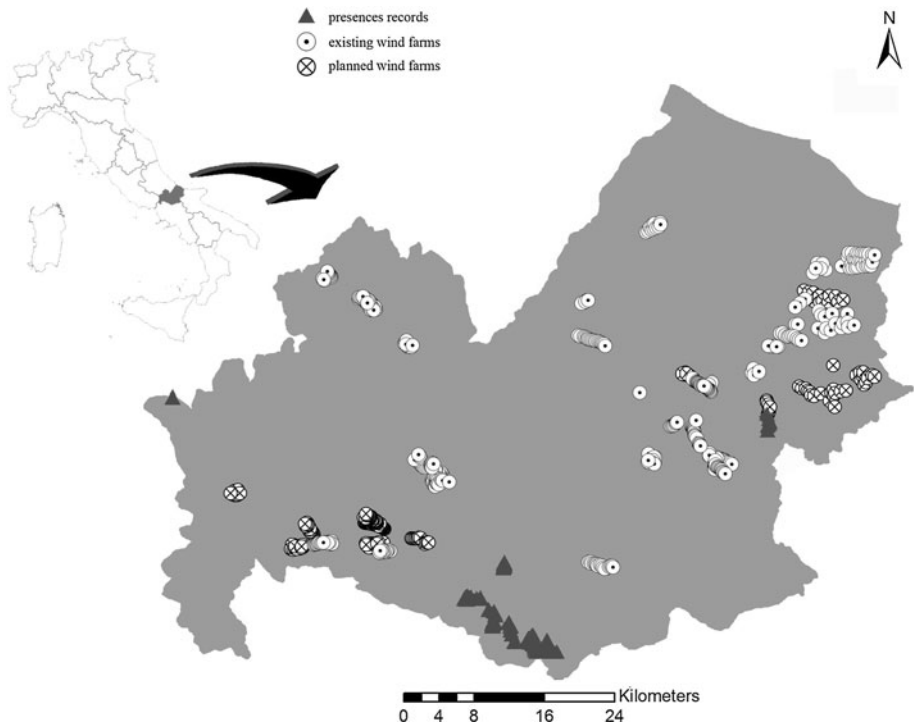


Fig. 1 Locations of the bat presence records and existing or planned wind farms in the Molise region (central Italy) used for modelling the impact of wind turbines on bats in this study

2006; Elith et al. 2006, 2011). For species recognition, we used the program BatSound 4.1. (Pettersson Elektronik AB, Uppsala, Sweden) to generate oscillograms, spectrograms, and power spectra, selecting one to three echolocation calls per sequence. When detected, social calls were also used for identification (Russo and Jones 2000; Russo et al. 2009). For the time-expanded recordings, we used a sampling frequency of 44.1 kHz, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis. The D1000X recordings were files generated with a sampling frequency of 384 kHz. Echolocation calls were identified by applying the classification functions described by Russo and Jones (2002), and social calls were identified according to Russo and Jones (2000) and Russ (1999).

Species distribution models

We considered three map layers as proxies of the environmental variables assumed to represent the relevant ecological requirements of the foraging habitat of the two species (Russo and Jones 2003; Rodrigues et al. 2008), i.e., the Digital Terrain Model at a 30 m spatial resolution (proxy for the altitudinal range), the Corine Land Cover map (CLC, European Commission 1993) 1:100,000 scale (Ciancio 2003) (proxy for vegetation types), and a hydrographic map with 30 m spatial resolution (proxy for freshwater bodies). The Molise region maps were obtained from (<http://cartografia.regione.molise.it/mapserver.html>). The wind farm locations of both existing and planned wind turbines were supplied by the environmental department of the Molise region. The models were developed using

the entropy distribution modelling software MAXENT 3.3.3e, a machine learning method that estimates the distribution of a species using only presence data (Phillips et al. 2004, 2006). This algorithm generally performs better than other similar techniques in predicting species distributions (Elith et al. 2006; Heikkinen et al. 2007) and also works with small datasets (Phillips and Dudík 2008). The algorithm applies the maximum entropy principle to an ecological context (Jaynes 1957), estimating the species distribution probability in such a way as to satisfy a set of constraints derived from the environmental conditions at the species' presence sites. These constraints ensure that the expected value of each environmental predictor falls as close as possible to the empirical mean of that predictor measured over the presence records (Phillips et al. 2004, 2006; Elith et al. 2006). Among all the possible distributions that satisfy the constraints, the algorithm chooses the one closest to the uniform, thus maximizing entropy. To allow a good performance of the maximum entropy algorithm, we converted the categorical layers (CLC and hydrography) into two continuous layers, according to Ficetola (Ficetola et al. 2010). Initially, the categorical CLC map was reclassified including, for computational reasons, only those Corine categories thought to be highly suitable for the studied species i.e., pastures (2.3.1), complex cultivation patterns (2.4.2), broad-leaved forests (3.1.1.), and inland water (5.1.1) (Fig. 2). On the resulting map, a focal statistic was performed to specify a circular neighbourhood of 1 km radius and to calculate the total number of cells (sum) in the neighbourhoods of each cell in the map. The hydrographic map was transformed in a

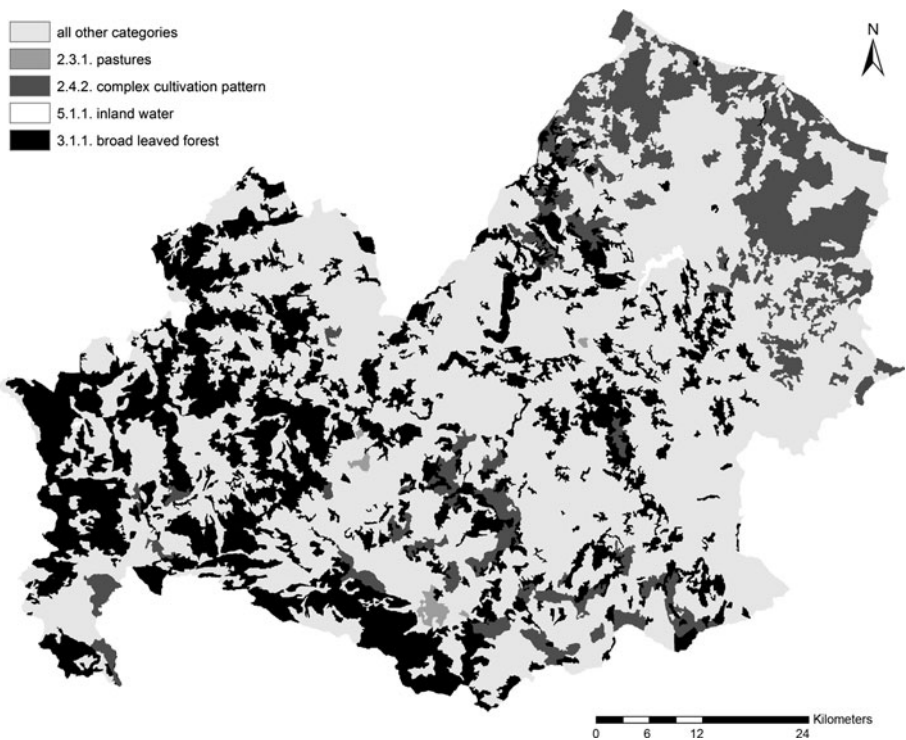


Fig. 2 The Corine land cover categories used to develop the inferential models for the Molise region (central Italy)

continuous layer by replacing the Euclidean distances of each pixel from any water course. These analyses were performed using ArcGis 10.0 (ESRI, Redlands, CA, USA).

We retained the default MaxEnt settings, with the exception of “number of replicates” and “default prevalence”. We set the software to split the occurrence data randomly into two subsets, with 70 % of the records used to train the model and the remaining 30 % used to evaluate its predictive power. This step was replicated 100 times, each time randomly selecting different 70–30 % portions of the occurrence data. The predictive power of the models was evaluated by calculating the area under the receiver operating characteristic curve (AUC) (Swets 1988), which represents a measure of the capability of the model to discriminate between presence and background data (Phillips et al. 2006). Because the species are quite easy to detect in typical presence sites, the value of “default prevalence” was set to 0.6 instead of 0.5 (default option) (Elith et al. 2011).

Impact assessment at the landscape level

To assess the overall impact of wind farms on the two bat species, the MaxEnt outputs were converted into binary maps (1 = suitable and 0 = non suitable), choosing the 10th percentile of the distribution of the probability of occurrences as the threshold (Phillips and Dudík 2008; Ficetola et al. 2009; Ficetola et al. 2010). We are aware that there is still no consensus on the “best” threshold to use and that the choice of a suitable threshold may have a great effect on the resulting probability map (Liu et al. 2005; Jimenez-Valverde and Lobo 2007). However, the 10th percentile threshold is considered to offer a highly conservative estimate of a species’ tolerance to each predictor in complex environments and for small datasets of species occurrences available for calibration (less than 25) (Pearson et al. 2007; Ficetola et al. 2009; Raes et al. 2009; Rebelo and Jones 2010; Zhu et al. 2012; Santos et al. 2013; Bosso et al. 2013; Svenning et al. 2008; Jarnevich and Reynolds 2011). The binary maps were then combined to identify the foraging areas suitable for both species. To evaluate the foraging habitat alteration caused by wind farms, the combined map was overlaid with that containing the location of existing and planned turbines, each buffered at 150 m. The buffer size was defined by considering the area of risk of collision and habitat loss around each turbine (Arnett 2005; Rodrigues et al. 2008). Alterations in the landscape patterns were explored through landscape indices computed on the SDM maps that included and omitted wind farms using FRAGSTATS version 3.3 (McGarigal and Marks 1995). The analyses were performed on three types of suitable feeding areas: suitable only for *P. pipistrellus*, suitable only for *N. leisleri*, and suitable for both species. After recording the coverage of each class in hectares (CA) and the total number of patches (NP), we focused on four class metrics reported to be ecologically meaningful (Riitters and O Neill 1995) and useful to describe habitat spatial patterns: largest patch index (LPI), mean patch size (MPS), area weighted mean shape index (AWMSI), and aggregation index (AI) (ESM1 see the supplementary material for details and formulae). The NP, MPS, and LPI metrics were selected because they are strongly related to habitat fragmentation (Forman 1995; Batistella et al. 2003), that is, the breaking up of one large suitable area into many smaller patches (Forman and Godron 1986; Shafer 1990; Fahrig 2003). The largest patch index (LPI) quantifies the percentage of total landscape area comprised by the largest patch of the suitable class and decreases in fragmented landscapes (Batistella et al. 2003). MPS is the average of the size in ha of all patches and corresponds to the ratio between the total area covered by each category and the number of patches in that suitability class; it decreases with landscape fragmentation. AWMSI measures the complexity of a suitable patch shape compared to a standard shape, which in raster format attains its minimum

value (AWMSI = 1) for squares and increases for irregular and elongated shapes. We computed AWMSI because of its ability to distinguish between the large, round-shaped patches that are characteristic of well-preserved habitats and small, irregular patches, which often dominate in disturbed landscapes (for a review, see Haines-Young and Chopping 1996). AI describes the adjacency of habitat “cells” (He et al. 2000) and ranges between 0 (when habitat distribution is maximally disaggregated; no adjacencies between cells of the same class) to 1 (when the landscape is totally homogenous). AI was used to describe habitat connectivity (Rutledge 2003). Lastly, considering the important role of forests as roosting and foraging habitat for bats (Grindal and Brigham 1999; Waters et al. 1999; Morris et al. 2010; Russo et al. 2007; 2010), we analyzed the interference caused by wind turbines situated close to forest edges. Interference was evaluated by the number of 150 m ranges of influence of wind turbines within 200 m from a forest edge (Rodrigues et al. 2008). The ranges of influences were created using the buffer distance function of ArcGis 10.

Results

Species distribution models

The Species Distribution Model for *N. leisleri* was supported by a 0.83 AUC value; the probability of presence for this species in the entire region ranged from 0 to 0.86. The generated SDM showed that 42 % of the regional territory is suitable for *N. leisleri* and that suitable areas are concentrated in the western part of the region (Fig. 3b). For *P. pipistrellus*, we obtained a 0.80 AUC and a probability of presence of 0–0.86. The *P. pipistrellus* foraging habitat covers 50.1 % of the regional territory, whereas it is concentrated in the western area for *N. leisleri* (Fig. 3a). Figure 3 shows the maps derived from the conversion of the MaxEnt outputs into binary maps using the 10th percentile of the distribution of probability of occurrence as a threshold: 0.26 for *P. pipistrellus* (Fig. 3a) and 0.19 for *N. leisleri* (Fig. 3b). The foraging areas suitable for both species represent 41.1 % of the regional territory (Fig. 3c), whereas only 0.5 % was suitable only for *N. leisleri* and 18.2 % for *P. pipistrellus* (maps not shown).

Impact assessment at the landscape scale

The 150 m radius circular buffer around each turbine overlaid to the combined SDM (Fig. 4) revealed that 66.7 % of the existing turbines and 51.5 % of the planned turbines were contained within the suitable feeding areas for both species, affecting 699 and 1,092 ha of foraging areas, respectively. The landscape pattern analyses performed on the three binary maps showed a change in all indices when wind turbines were added (Table 1). The NP and AWMSI indices showed that wind farms produced an increase in the number of habitat patches and in the complexity of patch shapes (Table 1). Although the mean patch size decreased as a consequence of both the existing and planned wind farms, the LPI index highlighted that the largest patches continued to represent the main component of the overall foraging area (Table 1). The degree of interference with forest edges showed that 13 % of the existing and 21 % of the total (planned + existing) 150 m turbine buffers overlapped with forest edges, affecting 2,660.67 and 3,141.68 ha of forest, respectively.

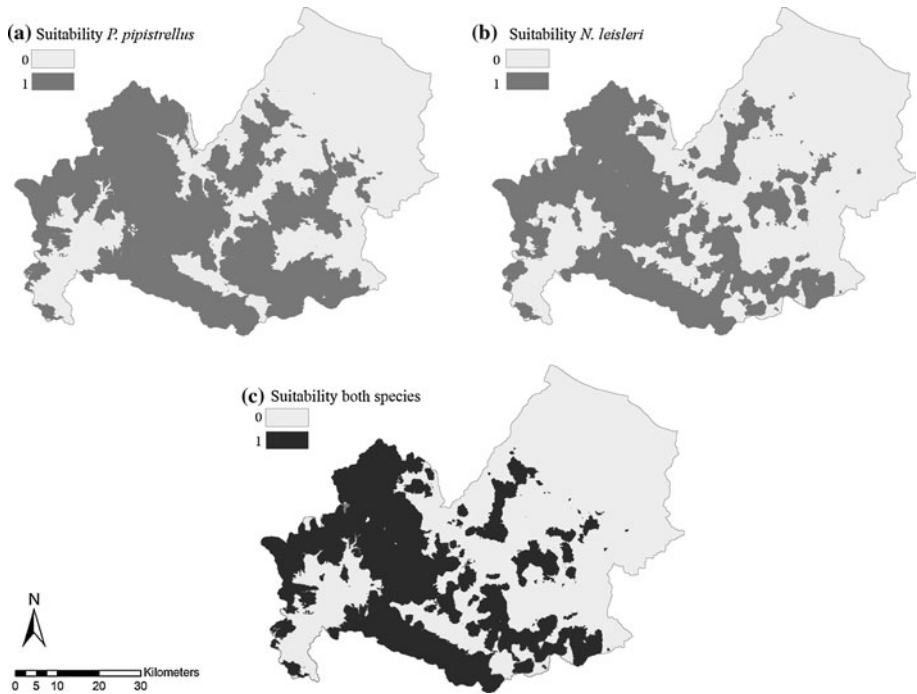


Fig. 3 Suitable foraging habitats for *P. pipistrellus* (a), *N. leisleri* (b), and both species together (c) in the Molise region (central Italy) obtained by converting the MaxEnt outputs into binary map using the 10th percentile threshold

Discussion

Modelling the impact of wind turbines on bats

Locating areas in which bats are exposed to increased risks of habitat loss and/or extra-mortality appears to be particularly important to mitigate the negative impact of wind farms on bats in a cost-effective manner (Santos et al. 2013). We performed a regional-based risk assessment for two sensitive bat species with an ecology that makes them representative of wider foraging guilds. The approach we followed may be easily extended to other bat species and for the exploration of more comprehensive species assemblages. It is important to emphasize that our approach may provide useful indications for the regional-scale planning of the spatial settings of wind farms and for orienting field surveys, thereby moving beyond the production of risk maps based on more subjective criteria or expert judgment (e.g., Cathrine and Spray 2009). Although our analysis does not incorporate mortality data, conservationists should accept that this information is still unavailable for many geographical areas and that the quality may be seriously flawed by incorrect or insufficient monitoring, even when available. Excessively long time intervals between surveys represent a typical factor causing bias in the results (Rodrigues et al. 2008) and may explain the apparently low mortality rates occasionally encountered. Clearly, approaches are needed that prove to be sufficiently effective, even when insufficient (or no) mortality data are available, as is the case for Italy (Ferri et al. 2011).

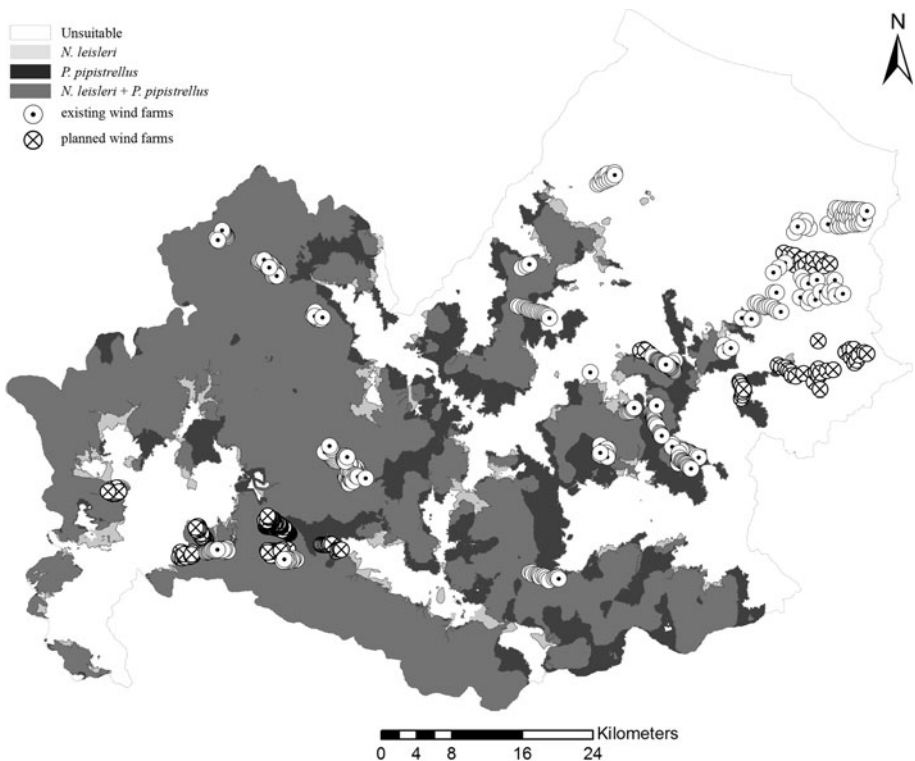


Fig. 4 Risk map for *N. leisleri* and *P. pipistrellus* developed for the Molise region (central Italy). Buffers (150 m radius) were traced around turbine sites and overlaid to the combined foraging habitat map for the two species. This map was produced by merging the binary maps obtained by the reclassification of the MaxEnt output considering the 10th percentile threshold

Moreover, modelling mortality may have its limitations because the mortality may be particularly high at sites near important roosts, yet such local peaks are unlikely to support inferences to other areas if detailed knowledge of roost location is unavailable.

Another constraint to modelling may be the limited availability of presence records. However, MaxEnt may effectively build representative models from limited presence datasets (e.g., Bosso et al. 2013), a fact that should make our modelling experiment easily implementable in many geographical areas and for many species for which limited distribution data are available. The models we developed for *N. leisleri* and *P. pipistrellus* proved to be statistically robust ($AUC \geq 0.80$; e.g., Swets 1988; Phillips et al. 2006; Phillips and Dudík 2008; Bosso et al. 2013) and highlighted that the western part of the Molise region was the most suitable for the foraging of both species. These species are known to prefer forest landscapes and traditional farmland (Waters et al. 1999; Russo and Jones 2003; Ruczynsky and Bogdanowicz 2005; Nicholls and Racey 2006; Sattler et al. 2007), both of which are well represented in that area. According to our model, over 50 % of the existing or planned wind farms are within areas suitable for both species. Johnson and Erickson (2003) remarked that the direct loss of habitat associated with the development of wind energy is smaller than that due to most other forms of energy development. Our results are in agreement with this observation because we found that only 1 % of the

Table 1 Modelled variation of landscape indices on a regional scale determined for the Molise region (central Italy) for two target bat species, *P. pipistrellus* and *N. leisleri*

Index	Species	No wind farms	% Variation existing wind farm	% Variation existing + planned wind farms
CA (ha)	<i>P. pipistrellus</i>	22,8007.04	-0.70	-1.00
CA (ha)	<i>N. leisleri</i>	18,8803.84	-0.65	-1.00
CA (ha)	Both species	17,6754.40	-0.69	-1.06
NP	<i>P. pipistrellus</i>	103	+7.76	+12.62
NP	<i>N. leisleri</i>	174	+4.02	+7.47
NP	Both	169	+4.14	+7.69
LPI (%)	<i>P. pipistrellus</i>	44.85	-0.67	-1.00
LPI (%)	<i>N. leisleri</i>	34.69	-0.55	-0.94
LPI (%)	Both species	32.17	-0.59	-1.02
MPS (ha)	<i>P. pipistrellus</i>	2,213.6	-7.86	-12.09
MPS (ha)	<i>N. leisleri</i>	1,085.0	-4.49	-7.88
MPS (ha)	Both species	1,045.88	-4.49	-8.12
AWMSI	<i>P. pipistrellus</i>	8.75	+8.50	+12.11
AWMSI	<i>N. leisleri</i>	6.41	+7.29	+11.89
AWMSI	Both species	7.30	+6.59	+10.73
AI (%)	<i>P. pipistrellus</i>	99.11	-0.08	-0.12
AI (%)	<i>N. leisleri</i>	99.11	-0.07	-0.11
AI (%)	Both species	98.91	-0.08	-0.12

Variations were calculated separately for existing wind farms as well as for both existing and planned ones CA class area, NP number of patches, LPI largest patch index, MPS mean patch size, AWMSI area weighted mean shape index, AI aggregation index. See text for definitions

foraging habitat for *P. pipistrellus* and *N. leisleri* available at a regional scale was actually lost. It is important to note that bats continue to forage at wind farm locations (e.g., Rydell et al. 2012), therefore feeding habitats are “altered” in terms of mortality risk being disproportionately increased at these sites. Wind farm facilities could act as ecological traps for bats because, by concentrating insect food sources, a turbine mast and rotor may represent a new, albeit deadly, attractive foraging opportunity (Ahlén et al. 2007, 2009; Horn et al. 2008; Rydell et al. 2010). Our study clearly showed the existence of significant landscape effects that are likely to affect bats, which have thus far been overlooked when bats are considered with regard to wind farm projects: a fine-grained analysis of the spatial pattern effects of turbines revealed that other factors in addition to the mere loss of habitat may have negative effects at a regional scale. The landscape indices showed that almost half of the suitable foraging area was included in a few large continuous patches that are slightly modified by the current wind turbines. This finding highlighted the relevance of these areas for the bat species we studied and the need to carefully consider these areas in wind farm planning. Although we did not note any macro-fragmentation effect caused by the wind turbines, we detected other alterations of the foraging habitat spatial patterns. The wind farms caused a change in habitat structure that acutely affected the suitable patches of limited size, leading to the formation of even smaller, discontinuous and isolated patches (as estimated by the increase in the number of patches and of the shape index and variation

in the LPI and AI indices). This process includes habitat incision, dissection, and perforation effects, all typical of the initial phase of habitat fragmentation (Forman 1995). The layers used in our study to develop SDMs showed the landscape spatial patterns observed prior to the development of wind farms, such that the changes highlighted by our analysis can be safely attributed to farm development. Of course, based on our dataset, we could not incorporate other human-related causes of fragmentation, such as expanding urbanization or farmland and forest management. We found that wind turbine development leads to an increase in the complexity of habitat patch edges, a process that deserves further attention. Indeed, many bat species forage along edges (Limpens and Kapteyn 1991; Grindal and Brigham 1999; Morris et al. 2010; Ethier and Fahrig 2011; Müller et al. 2012). Bats may also benefit from such linear elements as woodland edges for both foraging and commuting: such structures provide important insect habitat and may also serve as navigation landmarks (Grindal and Brigham 1999; Morris et al. 2010). Moreover, edges may provide shelter from predators or wind while reducing the energy expenditure associated with crossing more cluttered habitats (Verboom and Huitema 1997). According to our model, we estimated that 13 % of the existing turbines already interfere with forest edges and that this condition will also hold for another 8 % of the newly planned wind turbines. Given the importance of forests as habitats for bats, several other bat species in addition to those we targeted are likely to be jeopardized by this situation.

Implication for conservation

Overall, our study and that of Santos et al. (2013) showed that distribution models may offer a significant contribution to the prediction of a range of impacts at multiple spatial scales, from local habitat alteration to landscape fragmentation. These efforts can meet some of the research priorities highlighted in national or international documents regarding the consideration of bats in wind turbine development, including the EUROBATS guidelines (Rodrigues et al. 2008) and the bat conservation trust report for Britain (Jones et al. 2009). Our results also highlighted the value of a cumulative approach to identify the most important areas for bats, a crucial piece of information to sustainably locate wind farms or other widespread environmental stressors. We view our efforts as a first step toward the production of more comprehensive risk maps, which may be easily implemented using our approach, e.g., by adding more sensitive species to the model or by including other locally available information, such as the location of major roosts, occurrence of migration corridors, wind speed layers, or mortality statistics. However sophisticated, developing such models must not represent merely an academic exercise but rather offer a robust yet flexible and cost-effective tool that is applicable to a range of geographical and political situations. That is, a tool that is useful for assisting land managers in pre-construction planning, even when data and financial resources are limited or where environmental laws are ineffective in requiring comprehensive field surveys. In our example, even limiting the assessment to two representative species has made it possible to highlight the critical situation of the entire western sector of our study region and to detect a high risk of interference with forest edges, aspects thus far completely ignored by the local authorities responsible for wind farm development. This modelling approach may also be used to improve investigation on the impacts of wind farms, i.e., in comparing the cumulative impact on habitats or landscapes of different wind turbine locations at a regional scale and selecting the option that minimizes the negative effects on bat populations.

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CAPITOLO 8

BERARDO F., CARRANZA M.L., FRATE L., STANISCI A., LOY A. 2013.
Seasonal Habitat Preference by the Flagship *Testudo hermanni*. Implications for the
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Abstract: In this study, we assessed the seasonal selection of European Union habitats of interest by the flagship species *Testudo hermanni* in a well-preserved sand dune landscape on the Italian Adriatic coast. Nine tortoises, five males and four females, were fitted with transmitters and monitored for an entire season of activity. The eight EU habitats present in the study area were surveyed and mapped at a fine-scale using GIS. The seasonal preferential use or avoidance of each habitat was tested by comparing, through bootstrap tests, the proportion of habitat occupied (π_{Th}) with the proportion of available habitat in the entire landscape (π_{L}). The analysis of 340 spatial locations showed a marked preference for the Cisto-Lavanduletalia dune sclerophyllous scrubs (EU code 2260) and a seasonal selection of *Juniperus macrocarpa* bushes (EU code 2250*), wooded dunes with *Pinus* (EU code 2270) and mosaic of dune grasslands and sclerophyllous scrubs (EU codes 2230, 2240, 2260). Seasonal variation of habitat preference was interpreted in light of the different feeding, thermoregulation and reproductive needs of the tortoises. Our results stress the ecological value of EU coastal dune habitats and suggest prioritization of conservation efforts in these ecosystems

Seasonal habitat preference by the flagship species *Testudo hermanni* and implications for the conservation of coastal dune EU habitats

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Abstract. In this study, we assessed the seasonal selection of European Union habitats of interest by the flagship species *Testudo hermanni* in a well-preserved sand dune landscape on the Italian Adriatic coast. Nine tortoises, five males and four females, were fitted with transmitters and monitored for an entire season of activity. The eight EU habitats present in the study area were surveyed and mapped at a fine-scale using GIS. The seasonal preferential use or avoidance of each habitat was tested by comparing, through bootstrap tests, the proportion of habitat occupied (p_iTh) with the proportion of available habitat in the entire landscape (p_iL). The analysis of 340 spatial locations showed a marked preference for the *Cisto-Lavanduletalia* dune sclerophyllous scrubs (EU code 2260) and a seasonal selection of *Juniperus macrocarpa* bushes (EU code 2250*), wooded dunes with *Pinus* (EU code 2270) and mosaic of dune grasslands and sclerophyllous scrubs (EU codes 2230, 2240, 2260). Seasonal variation of habitat preference was interpreted in light of the different feeding, thermoregulation and reproductive needs of the tortoises. Our results stress the ecological value of EU coastal dune habitats and suggest prioritization of conservation efforts in these ecosystems.

Keywords: bootstrap, central Italy, Habitats Directive (92/43/CEE), habitat selection function, resampling method, Hermann's tortoise

Introduction

Coastal dunes represent extremely interesting environments both from an ecological and a landscape perspective (Van der Maarel 2003), but at the same time they are among the most fragile and threatened ecosystems worldwide (EEA 2008). Coastal ecosystems are particularly vulnerable to climate variability and to coastal erosion, and in recent years they have undergone consistent transformations due to urban expansion, agricultural and afforestation spread, and industrial and harbor development (Schlacher et al. 2007). The degradation and loss of the littoral landscape has concerned all coastal countries of the European Union and is particularly striking in the Mediterranean (Curr et al. 2000). For this reason, sandy coastal vegetation types are of most concern among EU directive habitats (European Commission, 2007), and most of the sand dune coastal fauna is included as threatened or endangered in the IUCN Red List (IUCN 2014). Some of these endangered species could act as flagship species, driving public awareness on conservation issues of the coastal dunes. Among these, the land tortoises (family *Testudinidae*) are the best candidates for coastal dune conservation, as they are charismatic and appealing to the target audience (e.g., EU Life Projects), are often endemic (Mazzotti 2006) and symbolize the uniqueness of the coastal dunes to foster a sense of local pride (Del Vecchio et al. 2011).

Specifically we focused on the Hermann's tortoise *Testudo hermanni* (Gmelin 1789) endemic to northern coastal Mediterranean and, the only indigenous species of Testudinidae found in Italy. *Testudo hermanni* is listed as endangered in the IUCN Red Data Book (IUCN 2014) and is threatened by the degradation and destruction of its habitat, especially in coastal areas (Mazzotti 2006; Cheylan 2011). This species is strictly protected by the Bern Convention and the European Habitat Directive (92/43 EEC, Annex II, IV), while the international trade of the species is regulated by the Washington Convention (CITES, App. II, C1). It is common in coastal areas of western central Italy; it is less common in the eastern coast and hilly landscapes (Mazzotti 2006).

The Hermann's tortoise occurs in Italy with two subspecies: *T. h. boettgeri*, mostly found along the northern Adriatic coast (Mirimin et al. 2004), and *T. h. hermanni*, prevailing along the Tyrrhenian coast. Hermann's tortoises are located in the xeric areas of the Mediterranean characterized by thermo and meso Mediterranean climate (Cheylan et al. 2011), mainly in the littoral pinewoods, coastal dunes, Mediterranean scrub, and garigues (Arnold and Burton 1985; Capula 1998; Filippi et al. 2010; Rugiero and Luiselli 2006). Most studies regarding the eco-ethology of *T.*

hermanni have been conducted in southern France (Cheylan 1981; Huot-Daubremont 1996), Greece (Hailey, 1984; Wright et al.1987) and Italy (Loy et al. 2007, Loy and Cianfrani, 2010).

Habitat preference of this species has been investigated in a variety of habitats, from coastal dunes (Calzolari and Chelazzi, 1991, Del Vecchio et al. 2011) to rural landscapes (Rozyłowicz and Popescu, 2012) while habitat preference studies accounting of the seasonal utilization of the different vegetation types are still necessary (Bertolero et al. 2011).

In this study, we explored if, how, and when the EU coastal dune habitats (*sensu* Habitats Directive 92/43/CEE) are used by Hermann's tortoises in a well-preserved coastal dune system of the Italian peninsula. Radio telemetry data and fine-scale vegetation habitat mapping were used to address the following questions: a) Is each EU habitat used differentially by Hermann's tortoises? b) Is there any seasonal variation in this utilization pattern? c) How does each habitat contribute to the ecological requirements of the tortoises?

We assumed that the utilization by tortoises of the EU habitat dune mosaic is not homogeneous but varies through space and time. By linking the EU coastal habitats to the survival of the flagship Hermann's tortoise, we contribute to the prioritization of conservation in this fragile and highly vulnerable ecosystem.

Method

Study area

The study area is part of the eastern coast of southern Italy (Molise region, Fig. 1). The Molise coast stretches for 30 km along the Adriatic Sea and is mainly composed of sandy beaches. Recent dunes (Holocene) occupy a narrow strip along the seashore. They are not very high (less than 10 m high) and are relatively simple in structure (usually only one dune ridge) (Acosta et al. 2009). In the dune profile, abiotic conditions vary greatly, moving along the sea-inland gradient. Under natural conditions, the vegetation zonation follows this ecological gradient, ranging from pioneer annual communities on the beach to Mediterranean macchia on the landward fixed dunes (Carranza et al. 2008; Acosta et al. 2009; Malavasi et al. 2013). The Mediterranean macchia can be considered the most mature vegetation type on fixed dunes. The climate of the area is typically Mediterranean, with dry summers, mild and rainy winters, and frequent precipitation (Carranza et al. 2008). The mean annual temperature is 15,9°C, the mean minimum

temperature is 13,2°C, the mean maximum temperature is 18,6°C, and mean precipitations amount to 356,8 mm/year (Termoli meteorological station, 1971-2000).

Despite the fact that dune vegetation has been severely damaged and reduced along the whole Adriatic coast (Malavasi et al. 2013), the coastal dunes of Molise still host many EU Directive 92/43 habitats (Feola et al. 2011; Stanisci et al. 2014). The study area is included in the SCI IT7222217 - Foce Saccione - Bonifica Ramitelli and is part of the Long Term Ecological Research Sites (LTER_EU_IT20_003_T) (Drius et al. 2013; Stanisci et al. 2007).

Vegetation map

Based on high-resolution panchromatic aerial photographs (dated 2008) and an extensive field survey (Spring 2013), we produced a detailed map at a scale of 1:1000 of the coastal dune habitats in the study area. Particular attention was given to natural dune cover types that, according to Acosta et al (2005) and Malavasi et al (2014), were mapped in nine different types which enclose eight habitats of European conservation interest according to Annex I of the European Council Directive 92/43/EEC (1992, Table 1). All artificial areas were classified as *no habitat*. Vegetation map was verified through several field surveys (performed from 2010 to 2012 using a GPS) and presented a global accuracy of 0.77. For each vegetation type ecological information, such as shading capacity and vegetation phenology was also reported. While shading capacity summarizes the presence of densely vegetated dunes and open areas that can help tortoises with thermoregulation activities, phenology describes budding and flowering periods (Pignatti 1982), thus a temporal supply of trophic resources.

Radio telemetry

A radiotelemetric study of Hermann's tortoise was performed between October 2012 and January 2014. A total of nine specimens (five males and four females) were captured through visual encounters by random walking across the coastal zonation from the drift line to the foredunes. Tortoises were equipped with a VHF radio transmitter (Very High Frequency, SOPR 2380 Transmitters – Wildlife Materials Inc.). Animals were located by means of a receiver (model R-1000 Telemetry Receiver 148-152 MHz w/220 of the Communications Specialist) connected to a Yagi or Adcock antenna. The tortoises were immediately released at the capture site and were monitored once a day for seven - eight consecutive days from April to October (one radiotelemetric fix location per day). During the hibernation

1 period (from November to March), each animal was located once a month. Each monitoring session lasted two hours
2 and was shifted during the seven - eight days to cover the whole daily activity period (maximum duration of 15
3 hours).

4 5 6 *Data analysis*

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8 According to the most common definition, habitat corresponds to the resources and conditions present in an area that
9 produces occupancy by a given organism (Hall et al. 1997). Thus, the aim of habitat selection studies is to identify
10 the environmental characteristics that make a place suitable for a certain species. A series of techniques have been
11 developed for habitat selection, including compositional analysis (Aebischer et al. 1993), Jacobs index (Jacobs 1974),
12 and selection ratio (Manly et al. 1993).

13
14 In this paper, habitat selection was performed by comparing the proportion of habitat occupied by *T. hermanni* (p_iTh)
15 with the proportion of available habitat in the entire landscape (p_iL) (i.e., first order selection) through a bootstrap
16 procedure (Efron 1979). In particular, to calculate the proportion of habitat occupied by *T. hermanni* (p_iTh) within
17 each habitat type, we overlapped the radio locations map with a buffer radius of three meters on the habitat-type map.
18 We obtained a nine-dimensional habitat-type compositional vector for each radio location (i.e., the distribution of the
19 area of each fix location within the nine habitats). The choice of this radius was based both on the consideration of
20 the visual field of the tortoises, their movement speed (personal observations), and the scale of the habitat map. Then,
21 a vector of the occupied habitat-type composition for the whole study area was obtained as a sum of the single-fix
22 compositional vectors. For a given habitat i , p_iTh was calculated as the ratio between the total area occupied by radio
23 locations in said habitat and the sum of the single-location compositional vectors (i.e., total area occupied by all the
24 fixes in the whole habitats). Finally, we tested whether the proportional area of a given habitat i that has been
25 occupied by *T. hermanni* (p_iTh) is significantly different from the proportional area of the habitat i in the entire
26 landscape p_iL . In particular we compared S , that is the absolute difference between the occupied and available habitat
27 proportion, $S = |p_iTh - p_iL|$, with SB that is the bootstrapped value of S . $SB = |p_iB - p_iTh|$, where p_iB is the
28 bootstrapped proportional area of a given habitat type (i) occupied by *T. hermanni* obtained by resampling with
29 replacement (999 times) the nine dimensional compositional vectors (see for details Manly 2007).

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If the actual value of S is sufficiently extreme in comparison to the bootstrap distribution (SB), the null hypothesis (random selection) is rejected. P-values were computed as the proportion of bootstrapped values SB that were greater than the actual values of S. After identifying a habitat for which the null hypothesis was rejected (random selection, $p < 0.05$), we investigated whether a specific habitat was positively ($p_iTh/p_iL > 1$) or negatively ($p_iTh/p_iL < 1$) selected by tortoises (Manly et al. 2002). The analysis was performed separately for males and females, for the whole activity season (April-October), and on pooled data of males and females for each monthly session.

Results

Vegetation map

The map included eight vegetation types, some of them characterized by a mosaic of EU habitats. Table 1 reports a the correspondence of vegetation types with EU habitats and a detailed description in terms of cover, phenology and shading capacity. The mosaic of dune grasslands and sclerophyllous scrubs (*Malcolmietalia* dune grasslands-cod. 2230, *Brachypodietalia* dune grasslands with annuals-cod. 2240, *Cisto-Lavanduletalia* dune sclerophyllous scrubs-cod. 2260) was the most-represented vegetation type, covering 23% of the study area. The least-represented type was the shifting dunes along the shoreline with *Ammophila arenaria* (white dunes, cod 2120), which covers 2% of the study area.

Unfortunately, the easternmost sector of the study area was recently burned (July 2007) and vegetation regrowth proceeded fairly slow. In particular, the large shrubs typical of coastal dunes, such as *Juniperus spp* bushes (cod. 2250*), have not grown back (Del Vecchio et al. 2013).

Radio telemetry

The detailed description of dates and total locations for each of the nine tortoises (five males and four females) are shown in Table 2. Seven of the nine animals were tracked for the entire period of activity, i.e., from the end of hibernation to the new hibernation period and were monitored for at least seven days per month (codes: 1 - 2 - 3 - 4 - 6 - 7 - 8). Two female tortoises were lost after one and three months (codes: 9 - 5). We obtained a total of 340 radio

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6 locations from October 2012 to January 2014. The distribution of three m buffered locations is shown in Fig. 1. In
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8 2013, all tortoises were active from late April to early July as well as from late August to early November.

9 10 11 *Habitat selection*

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13 According to the bootstrap tests, most vegetation types were used differently by *T. hermanni* (Table 3). The results
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15 obtained for males and females for the entire cycle of activity showed a marked preference for the dune
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17 sclerophyllous scrubs (EU cod. 2260). In addition, males also showed a preference for *Juniper macrocarpa* bushes
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19 (EU cod. 2250*) and for wooded dunes with *Pinus spp* (EU cod. 2270*) (Table 3).

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21 A marked preference for the dune sclerophyllous scrubs (EU cod. 2260) was also evident throughout the activity
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23 period, with the only exception of the session run in June, when tortoises selected the annual dune grasslands (EU
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25 cod. 2230), the dune grassland and sclerophyllous scrubs mosaics (EU cod. 2230, 2240 and 2260), and the no habitat
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27 class. Moreover, we identified a preference for some habitats during specific periods. While shifting dunes along the
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29 shoreline (EU cod. 2120) were selected in April, *Juniper macrocarpa* bushes were selected in May as well as from
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31 August to April, and wooded dunes with *Pinus spp* (EU cod. 2270*) were preferred in August.

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33 Generally, tortoises showed a preference for natural land cover types, as *no habitat* areas were negatively selected in
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35 all seasons, with the only exception of the session run in June, when most radiolocations were recorded near the
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37 railroad tracks. Another negatively selected vegetation type was the mosaic of annual vegetation of drift lines and
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39 embryonic shifting dunes (cod.1210 – 2110). Finally, wooded dunes with *Pinus spp* (EU Cod 2270*) were used in
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41 proportion to their availability without any significant tendency based on habitat availability (p values > 0.05), with
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43 the exception of August, when they were positively selected.

44 45 46 47 **Discussion**

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49 Radio telemetry and fine-scale habitat mapping provided an accurate framework of habitat preference and avoidance
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51 by the Hermann's tortoises in the Adriatic costal dunes, through accurate and replicable selection functions (HSFs).

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53 The habitat preference analysis suggested that the Hermann's tortoises operate both positive and negative selection of
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55 the different coastal dune vegetation types and that this pattern varies according to the season.
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The tortoises used most of the coastal dune EU habitats throughout their activity period except for those characterized by low shading capacity (code 1210-2110 in Fig. 1) or recent disturbance (e.g., burned). They also avoid artificial and agricultural areas (no EU habitats). Avoidance of the mosaics of annual vegetation of drift lines, embryonic shifting dunes and anthropogenic areas is most likely related to the scarcity of edible herbaceous vegetation and low density of shading plants. These habitats are, in fact, characterized by the prevalence of perennial graminoids with coriaceous leaves (Acosta et al 2006). The target species avoided burned juniper macchia (2230 with *Juniperus*), although edible vegetation is expected to increase after fire (new buds and young leaflets) (Mazzoleni and Esposito 1993). Thus avoidance could be due to the low availability of shrubs providing shelters against thermic stress and predators.

The most widely used EU habitats were the dune sclerophyllous scrubs (EU cod. 2260) and the *Juniper macrocarpa* bushes (EU cod. 2250*). These habitats are characterized by evergreen woody vegetation types growing on fixed dunes and quite sheltered from the harsh drift line conditions. Dune sclerophyllous scrubs are located in an intermediate position along the gradient from sea to inland and are characterized by a high richness of herbs and low bushes (Acosta et al. 2009). These habitats have a medium shading capacity and are rich in herbaceous edible species, thus they are likely used by tortoises for both feeding and thermoregulation. Similar preferences for clumped shading vegetation alternated with open areas were observed in both *T. hermanni* and *T. graeca* living in rural landscapes (Anadon et al. 2006; Rozyłowicz and Popescu 2012) and in the Tyrrhenian coastal dunes of Tuscany (Calzolari and Chelazzi 1991). Our results are also consistent with Blouin-Demers and Weatherhead (2001a, b), who postulated a general effectiveness of ecotones with patchy vegetation in providing optimal habitats for the thermoregulation of ectothermic species.

The observed seasonal utilization pattern of the different EU habitats by Herman's tortoises may be related to the capacity of each vegetation type to satisfy temporal variations in the thermoregulation, feeding, reproductive and breeding requirements of the species. For instance, the preference for the drift line and embryonic shifting dunes habitats (1210-2110) in early spring likely corresponds to the thermoregulation requirements of tortoises at the onset of activity after the winter hibernation. Indeed, during the first period of the activity cycle after hibernation, tortoises spend much of their active time basking to reach their optimal temperature (Cheylan 1981). This hypothesis is supported by local climatic data that indicate an average temperature of 16°C in April, corresponding to the minimum temperature of activation for tortoises (Huot-Daubremont 2002).

1 During the early summer, the Hermann's tortoise reaches the maximum peak of its activity (Cheylan 1981). The
2 preference for both annual dune grasslands (cod. 2230) and the mosaic of dune grassland with sclerophyllous scrubs
3 (cod. 2230, 2240 and 2260) in June is likely linked to the various activities performed in this season, such as feeding,
4 egg laying, and mating (Cheylan,1981, Wright et al. 1987).

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6 The need to escape the high summer temperatures at the onset of the pre-estivation phase (Cheylan 1981; Hailey
7 1984; Swingland and Stubbs 1985; Meek 1988 and Huot-Daubremont 1996) is the main factor that most likely
8 influenced the exclusive preference for the close vegetation of sclerophyllous scrubs (EU cod. 2260) observed in
9 July. From this period onwards, the open foredune habitats (Eu cod. 1210/2110; 2120; 2230 with *Juniperus*, 2230;
10 2230, 2240, 2260) were no longer selected due to both the heat and the reduced food availability, as the growing and
11 flowering stages are over and the plants are dry and leathery (Acosta et al. 2006).

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13 The increased need of protection from heat and the reduced metabolism during the estivation phase may explain the
14 exclusive preference for the sclerophyllous scrubs (EU cod. 2260) and the *Juniper macrocarpa* bushes (EU cod.
15 2250*) from August onwards. Only during August did tortoises select the wooded dunes (cod. 2270*), most likely in
16 search of coolness provided by the high shading capacity of the pinewoods. Moreover, in the late summer, occasional
17 rainfalls allow the growing of low herbs in the small gaps among the evergreen bushes, providing additional trophic
18 resources within these habitats.

37 **Final remarks and conservation implications**

38
39 This work contributed to our knowledge of coastal dune ecological processes and functioning. We focused on the
40 relationships between the flagship species *Testudo hermanni* and the coastal EU habitats. Our results confirmed that
41 climate and behavioral patterns play a crucial role in habitat use and preference by ectothermic species, especially
42 tortoises (Hailey et al. 1984; Meek 1984 and 1988; Pulford et al. 1984; Chelazzi and Cazolai, 1986; Carretero et al.
43 1995; Huot-Daubremont et al. 1996, Couturier et al. 2014). Hermann's tortoise broadly selected patchy areas where
44 close vegetation alternates with open areas with a specific seasonal pattern that was assessed here for the first time.

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46 The sustainable management of sclerophyllous scrubs (EU cod. 2260) and the *Juniper macrocarpa* bushes (EU cod.
47 2250*) may play a predominant role in the conservation of the Hermann's tortoise. Indeed, these habitats could act as

a type of “umbrella habitat,” as their protection triggers a sort of "chain protection" from the individual habitat to the entire landscape (Heywood 1995).

1 These habitats should be preserved not only for their intrinsic floristic value (Acosta et al 2009, Feola et al 2011) but
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3 also as providers of critical resources for the endangered tortoises. Conservation efforts toward these habitats could
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5 benefit from the presence of tortoises as a flagship species that could drive public interest and awareness toward these
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7 often neglected vegetation communities (Heywood 1995; Simberloff 1998). In addition, as tortoises are seed
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9 dispersers, their conservation contributes to the preservation of local flora (Jerzolimiski et al. 2009).
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13 Our analyses also revealed a seasonal utilization pattern of the EU habitats by tortoises across the coastal dune
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15 zonation. This temporal pattern is likely related to the specific capacity of each vegetation type to satisfy the
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17 thermoregulation, feeding, reproductive and breeding needs in different periods of the year. These outputs stress the
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19 importance of preserving the complete sequence of natural dune habitats, whose functionality is ensured by the
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21 integrity of its vegetation mosaic (Drius et al. 2013).
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Captions of figures and tables

Figures

1 Figure 1: Detailed vegetation map of the study area and radio locations. The profile diagram indicates the sequence
2
3 along the sea inland gradient of the mapped EU habitats. For codes see Table 1.
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Tables:

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11 Table 1: Detailed description of vegetation types along with the relative information concerning EU habitat types,
12
13 cover (percent of the landscape), vegetation shading capacity (percent of vegetation ground cover), and vascular
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15 plants dominant phenology (budding, presence of soft leaves, growing and flowering periods).
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20 Table 2: Description of radio tracked tortoises. For each animal, information concerning sex, number of radiolocated
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22 points and dates of the first and last radiotelemetric locations are reported.
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28 Table 3: Vegetation types and their degree of selection by the *Testudo hermanni*. For each type (see Table 2 for
29
30 vegetation description), the absolute differences between the occupied and available habitat proportions $S = |piTh-$
31
32 $piL|$, and the p values are reported. Values of $piTh/piL$ ratio > 1 indicate a positive selection; values of $piTh/piL < 1$
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34 indicate negative selection. N.S.= not significant at p values $> 0,05$.
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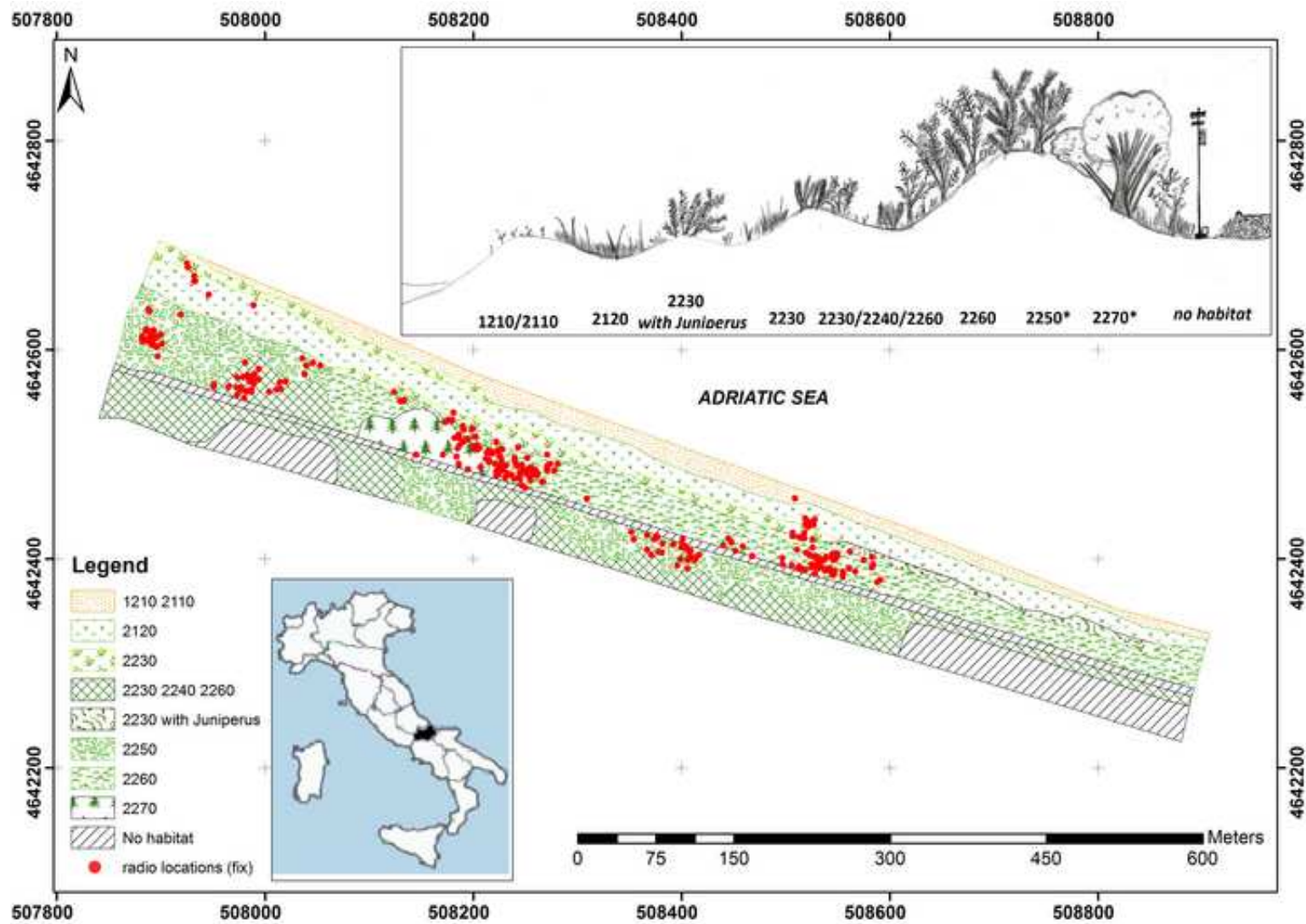
Description	EU code	Cover (%)	Shading capacity	Phenology
Annual vegetation of drift lines and embryonic shifting dunes (mosaic)	1210 / 2110	11,9	3,5	May-June
Shifting dunes along the shoreline with <i>Ammophila arenaria</i> (white dunes)	2120	2,22	15	May-June
Dune grasslands of <i>Malcolmietalia</i>	2230	4,45	0	April-June
Dune grasslands of <i>Malcolmietalia</i> with burned macchia	2230 (with macchia)	2,54	20	April-June
Dune grasslands and sclerophyllous scrubs mosaic: <i>Malcolmietalia</i> dune grasslands; <i>Brachypodietalia</i> dune grasslands with annuals and <i>Cisto-Lavanduletalia</i> dune sclerophyllous scrubs	2230 /2240 / 2260	22,87	40	April-August
Dune sclerophyllous scrubs of <i>Cisto-Lavanduletalia</i>	2260	4,94	50	evergreen
Coastal dunes with <i>Juniperus macrocarpa</i> bushes	2250*	22,08	70	evergreen
Wooded dunes with <i>Pinus pinea</i> and/or <i>Pinus pinaster</i>	2270*	3,93	80	evergreen
No habitat		25,07	0	

<i>Tortoise code</i>	<i>Sex</i>	<i>Date of the first location day/month/year</i>	<i>Date of the last location day/month/year</i>	<i>Number of location points</i>
1	M	18/09/2012	9/01/2014	45
2	F	18/09/2012	9/01/2014	44
3	M	19/09/2012	9/01/2014	43
4	M	25/04/2013	9/01/2014	46
5	F	28/04/2013	10/10/2013	22
6	M	28/04/2013	9/01/2014	44
7	M	28/04/2013	9/01/2014	45
8	F	28/04/2013	9/01/2014	45
9	F	26/04/2013	1/05/2013	6



	1210/2110	2120	2230 with Juniperus	2230	2230/2240/2260	2260	2250*	2270*	no habitat	
Cycle of seasonal activity (April – October) ♀ (female)	0,119 <0,001	0,022 <0,001	0,0007 0,915	0,013 0,464	0,228 <0,001	0,771 <0,001	0,112 <0,001	0,0393 <0,001	0,234 <0,001	S= piTh-piL p values
	0	0	N.S.	N.S.	0	16,59	0,4	0	0,06	piTh/piL ratio
Cycle of seasonal activity (April – October) ♂ (males)	0,11 <0,001	0,006 0,593	0,025 <0,001	0,026 0,002	0,066 0,003	0,096 <0,001	0,263 <0,001	0,103 <0,001	0,240 <0,001	S= piTh-piL p values
	0,07	N.S.	0,002	0,4	0,7	2,9	2,1	3,6	0,039	piTh/piL ratio
Session I (April) ♀ + ♂ (16°C)	0,089 <0,001	0,072 0,036	0,0251 <0,001	0,003 0,895	0,157 <0,001	0,520 <0,001	0,117 0,002	0,049 0,161	0,250 <0,001	S= piTh-piL p values
	0,2	4,2	0,009	0,9	0,312	11,5	0,466	N.S.	0	piTh/piL ratio
Session II (May) ♀ + ♂ (19°C)	0,119 <0,001	0,004 0,641	0,025 <0,001	0,014 0,574	0,103 0,009	0,267 <0,001	0,169 <0,001	0,040 0,229	0,238 <0,001	S= piTh-piL p values
	0	N.S.	0	N.S.	0,54	6,402	1,765	N.S.	0,044	piTh/piL ratio
Session IV (June) ♀ + ♂ (24°C)	0,119 <0,001	0,022 <0,001	0,025 <0,001	0,370 <0,001	0,161 <0,001	0,317 <0,001	0,241 <0,001	0,030 0,272	0,223 <0,001	S= piTh-piL p values
	0	0	0	5,960	3,402	0,134	0,477	N.S.	9,502	piTh/piL ratio
Session IV (July) ♀ + ♂ (26°C)	0,119 <0,001	0,022 <0,001	0,051 0,281	0,044 <0,001	0,086 0,187	0,303 0,001	0,083 0,297	0,084 0,206	0,250 <0,001	S= piTh-piL p values
	0	0	N.S.	0	N.S.	7,136	N.S.	N.S.	0	piTh/piL ratio
Session V (August) ♀ + ♂ (24.4.°C)	0,119 <0,001	0,034 0,309	0,025 <0,001	0,022 0,061	0,114 0,018	0,266 0,001	0,126 0,047	0,087 0,044	0,232 <0,001	S= piTh-piL p values
	0	N.S.	0	N.S.	0,499	6,374	1,572	3,216	0,071	piTh/piL ratio
Session VI (September) ♀ + ♂ (24°C)	0,119 <0,001	0,006 0,77	0,025 <0,001	0,021 0,242	0,08 0,125	0,259 <0,001	0,139 0,017	0,063 0,141	0,222 <0,001	S= piTh-piL p values
	0	N.S.	0	N.S.	N.S.	6,257	1,631	N.S.	0,109	piTh/piL ratio
Session VII (October) ♀ + ♂ (22°C)	0,119 <0,001	0,073 0,075	0,025 <0,001	0,007 0,767	0,123 0,006	0,268 <0,001	0,139 0,026	0,044 0,297	0,249 <0,001	S= piTh-piL p values
	0	N.S.	0	N.S.	0,458	6,434	1,629	N.S.	0,002	piTh/piL ratio

Figure
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CAPITOLO 9

STANISCI A., FRATE L., MORRA DI CELLA U., PELINO G., PETEY M., SINISCALCO C., CARRANZA M.L. 2014. Short-term signals of climate change in Italian summit vegetation: observations at two Gloria sites. *Plant Biosystems*. doi: 10.1080/11263504.2014.968232

ORIGINAL ARTICLE

Short-term signals of climate change in Italian summit vegetation: observations at two GLORIA sites

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Abstract

Short-term changes occurring in high mountain vegetation were analysed using the data from two Italian sites already part of the GLobal Observation Research Initiative in Alpine environments (GLORIA – central Apennines and southwestern Alps). The study focused on a set of floristic (endemics), structural (life forms) and ecological (thermic vegetation indicator) variables. Vegetation data were collected according to the GLORIA multi-summit standardized method during the last decade. The re-visitation revealed a moderate decrease in regional endemic flora and significant variations in structural and ecological parameters. The increase in caespitose hemicryptophytes in both sites, in suffruticose chamaephytes in the central Apennines and in rosette-forming hemicryptophytes in the southwestern Alps emerged, highlighting the rapid responses of the alpine vegetation to climate warming. The increase in perennial life forms is related with the expansion of graminoids and small woody plants. These life forms seem to be most suitable to face climate warming in Italian summits. The increase in the thermic vegetation indicator exceeds the mean European summits increment, and this is due to the expansion of thermophilic species. Short-term analyses with fine spatial and temporal resolutions are still necessary to improve our understanding concerning species behaviour in high-elevation ecosystems.

Keywords: *altitudinal ranks, climate warming, endemic species, life forms, thermic vegetation indicator, vascular plants*

Introduction

High mountain areas are hotspots of plant diversity and, in Europe, they host approximately 20% of the native flora (Médail & Quézel 1999; Aeschmann et al. 2013). This high plant diversity is related to a rich mosaic of different habitats above the forest line and to the presence of dispersal barriers between mountain ranges that facilitated speciation processes (Körner 1995). Europe shows a general north–south gradient of mountain endemism, increasing from boreal and temperate mountains to the Mediterranean summits (Faverger 1972). Many authors postulate that such gradient on endemic richness existed because, during the Pleistocene, mountains of the Mediterranean region were only partly glaciated and remained more isolated than northern European ones, which were extensively ice-covered

and whose margins were more connected to surrounding areas (Hughes & Woodward 2008). The isolation in Mediterranean high mountain environments, caused by the orographic discontinuity of cold areas (Chapin & Körner 1994; Körner 2003), gave rise to a peak of cryophilic endemic plants (Catonica & Manzi 2002). Indeed, on these mountains, cryophilic species are restricted to low open-vegetation on the highest summits, where they account for almost 90% of the total plant cover (Pauli et al. 2003; Casazza et al. 2005; Petriccione 2005; Stanisci et al. 2005, 2010; Fernández-Calzado et al. 2012; van Gils et al. 2012).

In the last 50 years, the mountain areas have experienced an increase in global average temperatures per decade of $0.13 \pm 0.03^\circ\text{C}$ and are projected to go through a higher rate of climate warming than most other regions of the world (IPCC 2013).

In addition, the decade 2001–2010 was the warmest one of the last century. Such climatic changes are affecting the flora and fauna at different spatial and temporal scales (Parmesan 2006). Concerning vascular plants, an increment in species richness in high mountain ecosystems is expected. Indeed, several studies have documented the upward shifting of thermophilic plants distribution both through long-term (Grabherr et al. 2001; Theurillat & Guisan 2001; Korner 2003; Walther et al. 2005; Nagy 2006; Cannone et al. 2007; Holzinger et al. 2008; Parolo & Rossi 2008; Britton et al. 2009; Engler et al. 2011; Palombo et al. 2013) and short-term time periods (e.g. Pauli et al. 2007, 2012; Erschbamer et al. 2011). Simultaneously, a decline of cryophilic endemic species is expected because of their specific habitat requirements, narrow distribution ranges and low capacity to modify their geographic distribution (Erschbamer et al. 2009). In particular, plant endemics of southern European high mountains, being distributed in small populations with low genetic diversity, are highly sensitive to global warming (Schwartz et al. 2006; Dirnböck et al. 2011; Fernández-Calzado et al. 2012).

Even if the analysis of changes in plant communities floristic composition (e.g. total species, focal species and key species) over time represents a sound indicator and predictor of environmental change, including climate warming, further plant structural and ecological parameters could be successfully used for the same purpose (Chiarucci & Bonini 2005; Carranza et al. 2012; Gottfried et al. 2012; Matteodo et al. 2013). For instance, the variation over time of life forms (Raunkiaer 1934) may provide a signal of ongoing ecological processes across different biogeographic and bioclimatic zones. In the context of climate change, identifying the life forms of expanding and contracting plant species is essential for predicting future changes in alpine vegetation (Illa et al. 2006; Vittoz et al. 2008; Epstein et al. 2013; Matteodo et al. 2013). In addition, the usefulness of plant species as ecological indicators of global warming has been demonstrated (Gottfried et al. 2012). Indeed, plants represent integrated expressions of the values of environmental variables that may strongly fluctuate in time and space (Dieckmann 2003). In particular, in order to quantify the warming effect or “thermophilization”, the analysis of the natural distribution of plant species along an altitudinal gradient (altitudinal ranks, ARs) could be very informative (Gottfried et al. 2012). Warming effect could be driven by two main mechanisms: filling process, given by an increment in the abundance of thermophilic outpost species, and moving processes (Grabherr et al. 1995), due to the immigration of thermophilic species from the lower altitudinal belts. Moreover,

the thermophilization could also derive from the disappearance of cold-adapted species (for details, see Gottfried et al. 2012).

In consideration of the factors noted above, the present work analysed the short-term changes occurring on biodiversity, structure and ecological patterns of plant communities on several Italian high-elevation summits. In particular, using the data series available from two monitoring sites belonging to the GLORIA network (GLORIA environments – <http://www.gloria.ac.at/>), we focused on changes of floristic (endemics), structural (life forms) and ecological (AR values and thermic vegetation indicator) variables. The results of 10-year monitoring of the Italian summits should clarify the following questions: (i) Has the endemic species frequency changed during the last 10 years? (ii) Has the frequency of life forms changed and, if so, are some of them more affected than others? (iii) Have plant communities revealed a “thermophilization” effect? (iv) Is there any evidence of expansion of lower elevation species (montane and treeline species) or, on the contrary, of the contraction of species of higher belts (alpine and nival)?

Material and methods

Study area

The study area has been set up within the GLORIA project network and is now also part of the European Long-Term Ecological Research (LTER) network (<http://www.lter-europe.net>). It includes two mountain sites (central Apennines and southwestern Alps – <http://www.lteritalia.it>), where the alpine vegetation has been monitored during the last decade. The temperature change between 2001 and 2012, downscaled to the investigated sites, was 0.61°C for central Apennines and 0.55°C for southwestern Alps (E-OBS database – Haylock et al. 2008) (Figure 1).

The Apennine site includes the alpine belt (from 2400 to 2790 m a.s.l.) of the Majella National Park. In the year 2001, three summits were selected to be monitored: Mt Femmina Morta at 2405 m a.s.l., Mt Macellaro at 2635 m a.s.l. and Mt Mammoccio at 2737 m a.s.l. This site is geologically characterized by a large limestone summit plateau, shaped by periglacial phenomena and by tectonic-karst depressions and surrounded by deep slopes (Giraudi 1998). The vegetation of the lower two summits is characterized by open grasslands with scarce vegetation cover (26% of the total surface) dominated by *Sesleria tenuifolia*, *Carex kitaibeliana*, *Helianthemum oelandicum* subsp. *alpestre*, *Androsace villosa*, *Iberis saxatilis*, *Arenaria grandiflora* and *Minuartia verna*. The vegetation cover of the highest summit is very low (15%) and the most frequent

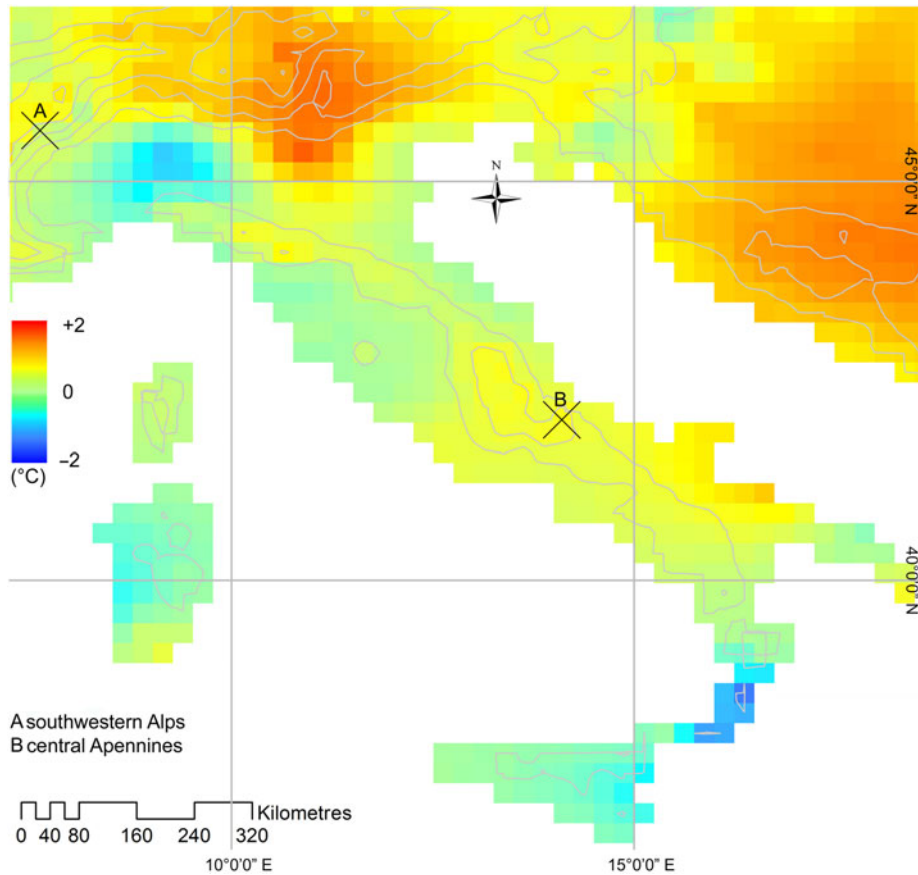


Figure 1. Location of the investigated high mountain GLORIA and LTER sites (crosses). The map represents the change in mean annual temperatures between 2001 and 2012. Temperature data were derived from E-OBS database with a resolution of 0.25×0.25 geographical degrees.

species are *Galium magellense*, *Cerastium thomasi*, *Viola magellensis* and *Alyssum cuneifolium*. The regional endemic species on the central Apennines represent 23% of the total sampled species. The dominant life form is hemicryptophytes, followed by chamaephytes, which progressively increase along the altitudinal gradient (Stanisci et al. 2005).

The Alps site is located in Aosta Valley (Mont Avic Natural Park – Champdepraz and in Valtournenche – Matterhorn Valley). In 2002, four summits were selected at the alpine belt: Col de la Croix at 2340 m a. s.l., Lago Balena at 2584 m a.s.l., Pra Pelat at 2790 m a.s.l. and Cime Bianche at 3014 m a.s.l. The Champdepraz area is characterized by the Piedmont Greenstone Calc schist, with outcrops of Greenstone alternate with volcanic sedimentary sequences while the Valtournenche area is formed by garnetiferous micaschists and calcschists. The vegetation of the lower summits consists of alpine open grasslands with the occurrence of shrubs, and some rare trees and the mean plant cover is low (39%). The most common species are *Carex fimbriata*, *Festuca varia*, *Juniperus communis* subsp. *alpina*, *Vaccinium uliginosum* subsp. *microphyllum*, *Carex curvula*, *Festuca halleri*, *Kobresia myosuroides*, *Leucanthemopsis alpina* and

Minuartia verna. In the highest summits, the vegetation is distributed in patches that cover < 14% of the total surface mainly occupied by debris. The dominant species are *Festuca quadriflora*, *Armeria maritima* subsp. *alpina*, *Silene acaulis* subsp. *bryoides*, *Minuartia sedoides*, *Saxifraga bryoides* and *Saxifraga oppositifolia*. In the site, the regional endemic species amounted to the 13% of the total species pool. Hemicryptophytes are dominant, followed by chamaephytes and geophytes (Geo; Aeschmann et al. 2012).

Data collection

On both the LTER sites, vegetation is monitored according to the GLORIA multi-summit standardized method (Pauli et al. 2004). The first data sampling was carried out in 2001 for central Apennines and 2002 for southwestern Alps and in 2012 both sites were re-visited (Table S1). For each summit, a 3×3 grid was established for each cardinal direction, at 5 m below the summit peak. In the four 1-m^2 corner plots (quadrants) of the grid, the species frequency was determined using a “frequency frame” divided into 100 subplots. In total, 7 summits and 112 permanent plots of 1 m^2 were surveyed. Species

identification and nomenclature follow the Flora Europaea (Tutin et al. 1964–1980). In order to minimize error linked to species identification, we filtered potentially misidentified species from the list, as described by Pauli et al. (2012).

Data analysis

We compared high summits vegetation between the two sampled dates, focusing on floristic information, structural parameters and ecological features. In order to investigate floristic changes, we analysed the frequency of endemic species; here, we considered as endemic only those species which are exclusive to a single mountain system (Pauli et al. 2012). Changes in the vegetation structure over time were examined using life forms. The following categories were revealed in the sampled flora: fruticose chamaephytes (ChF), reptant chamaephytes (ChR), suffruticose chamaephytes (ChS), cushion chamaephytes (ChC), succulent chamaephytes (ChSc), Geo, caespitose hemicryptophytes (HC), hemicryptophytes with rosette (HR), stalked hemicryptophytes (HS), biennial hemicryptophytes (HB), phanerophytes and nano-phanerophytes (Ph) and therophytes (Th). For the identification of endemic plant species and for the attribution of life forms, we referred to Pignatti (2005), Conti et al. (2005) and Aeschmann et al. (2004). Finally, ecological features were measured following the approach proposed by Gottfried et al. (2012) for the analysis of the response of mountain vegetation to climate change in Europe. We used vascular plant species as bio-indicators of thermophilization (*sensu* Gottfried et al. 2012). For each plant species, the AR value was assigned according to their specific altitudinal distribution. ARs vary from 1 to 6 as follows: AR 1, species with nival distribution centre; AR 2, alpine to nival species that do not descend under the treeline; AR 3, alpine centred species which do not descend to the montane belt; AR 4, alpine centred species that descend to the montane belt and species indifferently distributed from the treeline to the alpine; AR 5, species centred in the treeline ecotone or indifferently distributed from the montane to the alpine belts; AR 6, species which are montane centred or indifferently distributed from the montane belt to the treeline. Then, we calculated a thermic vegetation indicator (S) for each plot as follows: given p_{ij} as the frequency of species i in plot j , and AR_i the AR value of the species i , the thermic vegetation index is

$$S = \frac{\sum_{i=1}^n AR_i \times p_{ij}}{\sum_{i=1}^n p_{ij}}$$

It is important to note that an increment of S values (thermophilization) could be expected when

the frequency of species with high AR values (e.g. mountain species or thermophilic) increase or the frequency of species with low AR values decline (e.g. nival or cryophilic). In order to better investigate this ecological trend on each site, changes in AR frequency values were also assessed.

To analyse changes in the investigated parameters (endemics, life forms, S and AR values), we compared their frequencies in permanent plots between the two sampled dates (2001/2002 vs. 2012) using a paired test (Sokal & Rohlf 1981). Data were first tested for normality using a Shapiro–Wilk test (Sokal & Rohlf 1981). As the parameters (endemics, life forms, S and AR values) were not normally distributed, we chose the non-parametric Wilcoxon signed-rank test for paired samples (Wilcoxon 1945). The Wilcoxon signed-rank test (paired) uses the sign and the magnitude of the rank of differences between specific pairs of measurements and was used to determine whether the differences found between the median values of the first and the second data were significantly different from zero. Significance was evaluated at a 95% confidence level. All the analyses were performed by using the statistical software PAST (Hammer et al. 2001).

Results

During the last decade, the structure and ecology of high summits vegetation on the two Italian GLORIA and LTER sites changed significantly. Although the decline in the frequency of regional endemic flora was moderate (from 1541 to 1298 for central Apennines and from 1043 to 929 for southwestern Alps), structural and ecological parameters significantly varied for both of the analysed sites. A significant increase in HC frequencies was evident on both the mountain sites (Figure 2). In the southwestern Alps, HC total frequency significantly increased from 2589 to 2878 (+11%; $p = 0.0045$, $z = 2.836$), while in the central Apennines it increased from 963 to 1269 (+32%; $p = 0.0086$, $z = 2.625$). Moreover, in central Apennines, a significant increment of frequencies was found for ChS: they grow from 1701 to 2227 (+31%, $p = 0.011$, $z = 2.539$). On the southwestern Alps, significantly higher frequency values were evident for HR (Figure 2) ($p = 0.001$, $z = 3.254$) by about 20% (from 930 to 1114). On the other hand, a significant reduction of Geo, from 823 to 682 (–17%) was also observed on the southwestern Alps ($p = 0.0278$, $z = 2.200$). The thermic vegetation indicator (S) showed significantly higher values in 2012 as compared with the older data (Figure 3). For central Apennines, S increased from 3.61 to 3.72, with a mean increment of 0.11 ($p = 0.001$, $z = 3.056$), while for southwestern Alps S changed from 2.90 to

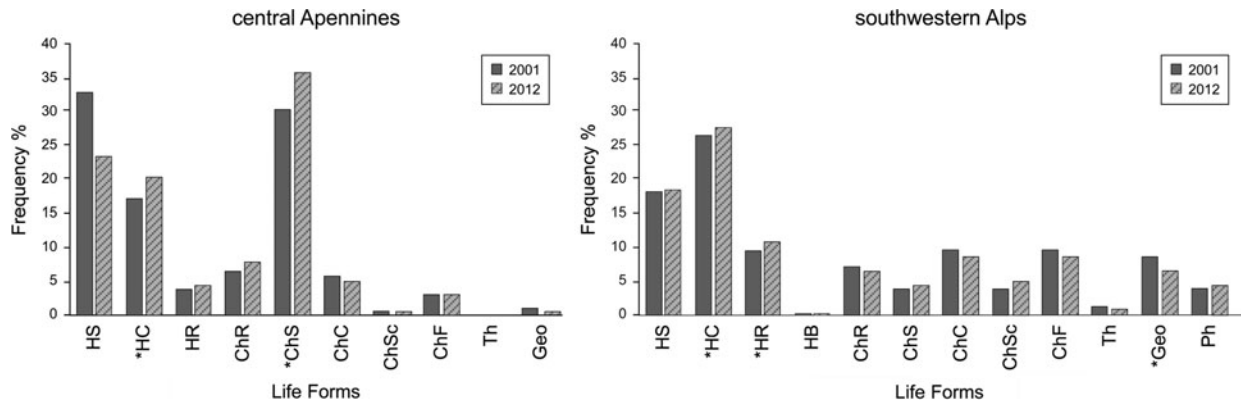


Figure 2. Changes in life form frequencies between the two analysed dates for both the investigated GLORIA and LTER sites. HS, stalked hemicryptophytes; HC, caespitose hemicryptophytes; HR, rosette hemicryptophytes; HB, biennial hemicryptophytes; ChR, reptant chamaephytes; ChS, suffruticose chamaephytes; ChC, cushion chamaephytes; ChSc, succulent chamaephytes; ChF, fruticose chamaephytes; Th, therophytes; Geo, geophytes; Ph, phanerophytes and nano-phanerophytes. Asterisks indicate significant differences ($p < 0.05$) according to the Wilcoxon signed-rank test (paired).

2.99 with a mean increment of 0.09 ($p = 0.031$, $z = 2.148$). In the central Apennines, the frequency of plant species with wide distribution (AR 4) and montane species (AR 6) showed a significant increase (AR 4: $z = 3.101$, $p = 0.001$; AR 6: $z = 2.117$, $p = 0.047$) (Table 1), while in the southwestern Alps the frequency of timberline species (AR 5) ($z = 2.668$, $p = 0.007$) increased.

Discussion

The weak short-term variation in regional endemics of the examined sites seems to be in line with the general theory according to which high-elevation

endemics are expected to decline under a warmer climate (e.g. Grabherr et al. 1995; Pauli et al. 2003; Schwartz et al. 2006; Dirnböck et al. 2011; Fernández-Calzado et al. 2012). Anyway, the weakness of the signal could be related to two main issues: (i) the homogeneous (micro-topographical and microclimatic) conditions which characterize the monitored sites and (ii) the limited inter-specific competitive pressure on the sampled harsh environment (Dullinger et al. 2007; Cavieres et al. 2014). Note that we have monitored only ridge and summit habitats vegetation, while the abovementioned theory is based on the entire flora present in the high-elevation mosaic (micro-topographical and microclimatic). Indeed, it is well documented that, beside the ridges, also scree and cliffs ecosystems host a rich pool of endemic species (Pauli et al. 2003; Casazza et al. 2005; Bragazza 2009; Stanisci et al. 2010; Fernández-Calzado et al. 2012). Taking into account that the manner in which endemic species respond to climate warming depends on their abilities to cope with micro-topographical situations (Gottfried et al. 1999), further studies addressing the behaviour of the endemic species on dolines, screes,

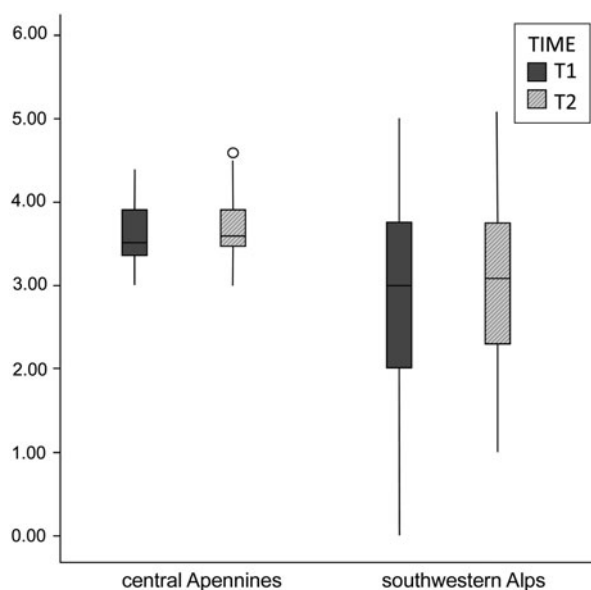


Figure 3. Box and whisker plots of the thermic vegetation indicator S. T1, first data sampling (2001–2002); T2, re-survey performed in 2012. Significant differences between the compared dates (Wilcoxon signed-rank test, $p < 0.05$) were evident for both the LTER sites.

Table 1. Changes in AR frequencies between the two analysed dates for both the investigated GLORIA and LTER sites.

AR	Central Apennines			South-Western Alps		
	Trend	z	p -Value	Trend	z	p -Value
1	–	0.457	0.665	–	1.010	0.321
2	+	0.145	0.892	+	1.939	0.052
3	–	1.623	0.104	+	1.677	0.094
4	+	3.101	0.001	+	1.026	0.031
5	+	1.430	0.158	+	2.668	0.007
6	+	2.117	0.047	+	1.000	0.317

Note: Significant p -values ($p < 0.05$), according to the Wilcoxon signed-rank test (paired), are in bold; +, increase; –, decrease.

cliffs, plateau and slope habitats are needed. Second, the weak variation of endemic species frequencies on ridge and summits could also be due to the extremely harsh environmental conditions (high wind speed and extreme temperature fluctuations) which, in these habitats, limit the inter-specific competitive pressure (Körner 2003; Gutiérrez-Girón & Gavilán 2010). At least for a short-term period of observation, the only endemic taxon showing a strong decline on central Apennines ridges is the steno-endemic *Viola magellensis*. Further studies are necessary to verify its trend over time in other high-elevation habitats where it was previously recorded, such as the stable scree on mild slopes (Blasi et al. 2005; Stanisci et al. 2010; van Gils et al. 2012).

As concerns vegetation structure, different “fingerprints” of the climate warming may be clearly detected across the GLORIA/LTER Italian summit habitats. The observed increase in HC frequencies is due to the expansion of graminoid species, characteristic of open grassland communities present in areas between the timberline and the alpine zone. Matteodo et al. (2013) detected similar trends, in a long-term analysis of the Swiss Alps and indicated the HC as particularly prone to face the ongoing processes of climatic change. Indeed, these graminoids have likely been favoured by their efficient vegetative reproduction and by their well-adapted leaves to support dry environmental conditions (Aeschmann et al. 2012). In addition, the observed rise in HR frequencies in the southwestern Alps site is probably due to their phenological characteristics. Indeed, as documented in an alpine area of Sweden, species with delayed flowering (e.g. HR) respond positively to global warming (Molau et al. 2005). Moreover, even though five types of chamaephytes are present in the central Apennines (ChR, ChS, ChC, ChF, ChSc), only the ChS show a significant increase. Such results pinpoint this life form as the most suitable one among the chamaephytes to face the warming effects. The observed increase in ChS is due to the expansion of the smallest woody plants in the area, such as *Arenaria grandiflora*, *Iberis saxatilis*, *Edraianthus graminifolius* and *Vitaliana praetutiana*. A similar increase in chamaephytes, mainly dwarf-shrubs, was also observed in the alpine grasslands (Vittoz et al. 2008) and in the arctic tundra (Myers-Smith et al. 2011; Epstein et al. 2013). The potential ability of ChS to respond in a warmer world should be verified through further comparative analysis including other LTER Mediterranean high summits. Overall, the observed trends of dominant perennial life forms (hemicryptophytes and chamaephytes) agree with the expected increment of vascular plant biomass described for arctic and alpine habitats (Cornelissen et al. 2001). In addition, our findings are supported by short-term models that forecast a

general increase in dwarf shrubs and in competitive clonal graminoids in alpine ecosystems (Erschbamer et al. 2009). Such structural changes may also have implications for other taxonomical groups, such as invertebrates and birds which have precise habitat breeding and foraging requirements (Nagy et al. 2003). Finally, in the southwestern Alps, the Geo significantly decrease. Note that only three species belong to this group (*Carex fimbriata*, *Lloydia serotina* and *Polygonum viviparum*) and they are present only at lower altitudes. Moreover, previous long-term research carried out in the Alps (Matteodo et al. 2013) evidenced very weak variations of Geo. To explain our results, we can simply speculate, at the present time, that Geo, being typical of wet microhabitats (Aeschmann et al. 2012), decreased during the last decade probably because summit habitats have become drier. Further research on the behaviour of Geo in the alpine belt ecosystems under different scenarios of global change is necessary.

The observed increment of the thermic vegetation indicator *S*, for both the investigated sites, is higher than the mean increment of *S* value recorded for all the European summits in the period 2001–2008 (Gottfried et al. 2012). This increase mostly reflects a filling process of thermophilic species already present at the alpine belt, rather than immigration of species from lower elevations. For example, in the central Apennines, the frequency of many species with wide distribution between timberline and alpine belt (AR 4) (e.g. *Sesleria tenuifolia* and *Arenaria grandiflora*), as well as montane species (AR 6) (e.g. *Carex humilis*) significantly increased. Similar results were reported by Petriccione (2005) using short-term phytosociological data. In particular, dry grasslands communities dominated by *Sesleria tenuifolia* and *Carex humilis* are favoured by the current climate warming, and are filling an increasing area on summit habitats. Such filling process could be related with the ability of this dry grasslands communities to live on windy ridges habitats above the timberline (Blasi et al. 2005; Di Pietro et al. 2008), where eolic sedimentation of fine particles give origin to shallow soils. At the same time, in the southwestern Alps, timberline species group (AR 5) (e.g. *Festuca varia* and *Juniperus alpina*) showed a consistent increase. Here, alpine grassland communities, dominated by *Festuca varia* and *F. halleri*, and alpine juniper shrubland have strengthened their presence in the last decade. Although a decline of cold adapted species could be expected, the current observed thermophilization is only due to the increase in thermophilic species frequencies. Similar evidence has emerged from previous studies concerning high-elevation ecosystems of Alps (e.g. Grabherr et al. 2001; Walther et al. 2005; Cannone et al. 2007; Pauli et al. 2007, 2012; Parolo & Rossi

2008; Erschbamer et al. 2011). Erschbamer et al. (2009) argued that increasing temperatures and prolonged growing seasons enhanced soil development and consequently promote the expansion of species from the lower altitudes.

Conclusion and final remarks

The analyses of short-term changes on biodiversity, structure and ecological patterns of plant species in Italian high-elevation summits suggest a rapid response of the alpine vegetation to the ongoing climate warming. Such findings agree with recent short-term studies at both continental (Gottfried et al. 2012; Pauli et al. 2012) and local scales (e.g. Cannone et al. 2007; Erschbamer et al. 2009; Vittoz et al. 2010). While studies based on centuries data series (e.g. Grabherr et al. 2001; Erschbamer et al. 2011; Matteodo et al. 2013) can help to identify large-scale patterns of change and can offer a benchmark for making global predictions over time (Matteodo et al. 2013), short-term analyses of ecological indicators with smaller spatial and temporal resolutions are still necessary to improve our understanding concerning species behaviour in a changing climate. The short-term analysis of LTER Italian summits clearly shows the ongoing increment of thermophilic and perennial plant species. This process is expected to further accelerate according to climate warming predictions.

It would be advisable to test our findings throughout the southern Europe mountain LTER sites, as they are excellent observatories to investigate the ecological responses to climate warming. Moreover, in order to increase our knowledge concerning the ecological processes caused by climate change in the high-elevation ecosystems, it could be useful to analyse the floristic structural and ecological features of each habitat of the high-elevation landscape mosaic, because the micro-topographical and micro-climatic heterogeneity may influence the specific dispersal pattern of each sensitive plant species, as well as their resilience and resistance.

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Supplemental data

Table S1. List of recorded species in the two dates (2001/2002 and 2012) for both the GLORIA sites (central Apennines and south-western Alps). Life forms, altitudinal rank and species frequency are also provided.

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CAPITOLO 10

Frate L., Carranza M.L., Garfi V., Di Febbraro M., Tonti D., Marchetti M., Ottaviano M., Santopuoli G., Chirici G. Spatially explicit estimation of forest age by integrating remotely sensed data and inverse yield modeling techniques. *iForest*, *Under Review*

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**Spatially explicit estimation of forest age by integrating remotely sensed data and
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Keywords:	K-nearest Neighbors; Mapping; Forest Inventory; Growing Stock; IRS LISS-III
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1 **Spatially explicit estimation of forest age by integrating remotely sensed data and**
2 **inverse yield modeling techniques**

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16 **Keywords:** k-nearest neighbors, mapping, forest inventory, growing stock, IRS LISS-III

17 **Running title:** Multi-source forest age mapping

18 **Abstract**

19 In this work we present an innovative method based on the application of inverse yield
20 models for producing spatially explicit estimations of forest age. Firstly, a raster growing
21 stock volume map was produced using the non-parametric k-Nearest Neighbors (k-NN)
22 estimation method on the basis of IRS LISS-III remotely sensed imagery and field data
23 collected in the framework of a local forest inventory. Secondly, species specific inverted
24 yield equations were applied to estimate forest age as a function of growing stock volume.
25 The method was tested in 128,000 ha of even aged forests in central Italy (Molise region).
26 The accuracy of the method was assessed using an independent dataset of 305 units from a
27 local standwise forest inventory. The results demonstrated that the forest age map was
28 accurate, the RMSE was 15.78 years (the 30% of the average real values), thus at least useful
29 for supporting forest management purposes, such as the assessment of harvesting potential,
30 and of ecosystem services. Thanks to the use of remotely sensed data and spatial modeling,
31 the approach we propose is cost-effective and easily replicable for vast regions.

32

33 **Introduction**

34 Forest ecosystems cover approximately 31% of the world's land surface and provide a wide
35 range of ecological, economic and socio-cultural services (FAO 2012). In forest ecosystems,
36 the tree and stand age are strongly related to ecosystem productivity (Chirici et al. 2011),
37 functionality (Pan et al. 2010) and services (Costanza et al. 1997), including carbon stock and
38 fluxes (Bradford et al. 2008, Pan et al. 2010) and regulation of the hydrologic cycle (Cornish
39 & Vertessy, 2001). In addition, forest stand age is a good indicator of forest biodiversity
40 (McRoberts et al. 2011, Burrascano et al. 2013, Seidling et al. 2014). Old trees are, in fact, an
41 important habitat for specialist forest species, such as birds (Conner & Dickson 1997), small
42 mammals (Ecke et al. 2002), bats (Russo et al. 2010), beetles (Lassauce et al. 2013), lichens,
43 fungi and bryophytes (Rambo & Muir 1998, McGee & Kimmerer 2002). Moreover, forest
44 stand age affects vascular plant diversity (Halpern & Spies 1995, Pitkänen 1997, Honnay et
45 al. 1998, Deconchat & Balent 2001). Therefore, it is important to identify the distribution
46 patterns of forest stand age to understand and balance the benefits and drawbacks of different
47 management scenarios which should be aimed at guaranteeing and maximizing ecosystem
48 services provisioning in time (Forest Europe 2011, Gamefeldt et al. 2013). The importance of
49 forest age is officially acknowledged by its inclusion in the set of 35 pan-European indicators
50 (MCPFE 2001) routinely used to monitor and assess sustainable forest management in
51 Europe (MCPFE 2002, MCPFE 2007, Forest Europe 2011). Consequently, accurately and
52 spatially estimating forest stand age is essential in order to support environmental assessment
53 and forest monitoring programs at a variety of spatial scales (Chirici et al. 2011).

54 The interest in the spatial reconstruction of the forest age patterns in Europe is demonstrated
55 by at least three recent studies. Bellassen et al. (2011) used the ORCHIDEE-FM process-
56 based vegetation model to reconstruct past age-class distributions in the period 1950 – 2000.
57 Seidl et al. (2011) used the matrix approach of EFISCEN (Schelhaas et al. 2007), developing

58 a simple age-class distribution backcasting method. Vilén et al. (2012) first compared the two
59 methods, reconstructing the forest age structure in Europe in the period 1950-2000. They
60 found that the simplest Seidl et al. (2011) method was more accurate than that one proposed
61 by Bellassen et al. (2011), and then introduced the use of the forest/non-forest map produced
62 by the Joint Research Centre of the European Commission (Pekkarinen et al. 2009) to
63 produce a low resolution (0.25°) forest age map.

64 Although the Vilén et al. (2012) study was extremely useful in depicting a potentially
65 dangerous decrease in the area of old forests in Europe, the achieved results were mainly
66 useful when aggregated at national level. The maps produced by Vilén et al. (2012) are in fact
67 very coarse in the spatial resolution and are the result of a highly simplistic assumption: the
68 homogenous distribution of all age classes over the forest area in a country. In other words,
69 the distribution of forest age classes available from National Forest Inventories of a given
70 country are considered invariant in all the forest area of that country.

71 The aim of our study is to propose a simple and straightforward method for the high-
72 resolution spatial estimation of forest age, integrating plot level forest inventory data with
73 remotely sensed imagery through the application of inverted yield-models. Such an approach
74 could be easily implemented to create the age structure at a given time, useful for a high
75 resolution application of the method proposed by Vilén et al. (2012) or other approaches
76 based on forest disturbances detection (Kennedy et al. 2010).

77 National forest inventories are the primary source of data for national and large area
78 assessments for international forest resource reporting (McRoberts et al. 2009). A
79 questionnaire developed within the COST Action E43 “Harmonisation of National Forest
80 Inventories in Europe: Techniques for Common Reporting” revealed that forest age is

81 currently estimated by the NFIs of 96% (out of a total of 22 investigated countries) (Chirici et
82 al. 2012a).

83 The provisioning of low cost and widely available remotely sensed data has added a spatial
84 component to forest attribute information acquired in the field by forest inventories via the
85 construction of maps. McRoberts & Tomppo (2007) presented a review of the methods used
86 for integrating remotely sensed data into NFI projects and according to the review from
87 McRoberts et al. (2010), the methods for constructing maps based on plot level NFI data can
88 be distinguished on the basis of several factors. Forest categorical (e.g. forest types) or
89 continuous (e.g. growing stock volume) variables can be estimated with supervised or
90 unsupervised approaches, the estimates can be provided at pixel level or for aggregated areas,
91 and parametric or non-parametric algorithms can be used.

92 Even if a large number of successful examples of application of parametric approaches exists,
93 for the estimation of forest variables, the non-parametric k-Nearest Neighbors (k-NN) method
94 successfully emerged as the most popular in the last years, at least when forest inventory data
95 is used. Commonly estimated forest variables include growing stock volume, forest type, and
96 commonly used remotely sensed variables include optical spectral bands, radar information
97 and, increasingly in the last decade, airborne laser scanning metrics.

98 With respect to the very large number of studies related to the spatial estimation of more
99 traditional forest variables (such as growing stock volume or biomass) a relatively low
100 number of experiences are available in literature for stand age estimation and mapping. Apart
101 from the already mentioned recent experiences presented by Bellassen et al. (2011), Seidl et
102 al. (2011) and Vilén et al. (2012) the relationship between remotely sensed reflectance and
103 stand age has been studied since the early '90s of the previous century (Nilson & Peterson
104 1991, Nilson & Peterson 1994). Stand age maps can be produced adopting multi temporal or

105 single temporal approaches. Multi temporal methods date forest stands accounting for the
106 years since the last natural or anthropogenic disturbance. Several examples of this so-called
107 temporal trend analysis (Kennedy et al. 2010), which is usually based on the integration of
108 optical satellite data and ancillary information, exist (see for examples Zhang et al. (2004) for
109 Canada and Pan et al. (2010) for North America).

110 Single temporal approaches are instead aimed at predicting the age of forest stands at a given
111 date without reconstructing past disturbances and thus using remotely sensed images acquired
112 on one date only. In these works, the stand age or the stand age classes are measured or
113 acquired in the field in a sample of the forest (in plots or in stands). When categorical age
114 classes have to be predicted and mapped, parametrics, such as the well-known maximum
115 likelihood (Nel et al. 1994), or non-parametric algorithms, such as neural networks (Kimes
116 1996), can be used. The spectral values of the different bands of a multispectral satellite
117 sensor (the Thematic Mapper of the Landsat satellites is the most common) are used as
118 predictors, as they are, or transformed through the calculation of vegetation indexes, or
119 manipulated with moving window filters. As for the multitemporal approach, predictors from
120 remotely sensed data are frequently coupled with ancillary layers, such as those created on
121 the basis of a Digital Elevation Model (Kimes 1996).

122 When, instead, contiguous values of forest age have to be estimated, the most common
123 approach is based on the use of the non-parametric k-Nearest Neighbor algorithm
124 (Holmström et al. 2003, Reese et al. 2003). In these cases, stand age is measured in sample
125 plots in the framework of a formal forest inventory.

126 In our opinion, this approach has two main limitations. Firstly, in forest inventories plot level
127 forest stand age is usually calculated by examining tree rings from cores of selected trees.
128 This can be an inexact process because in reducing measurement costs, only one or a few

129 trees may be cored in the field for each plot (Pan et al. 2010). Secondly, in Europe, several
130 definitions of stand age are adopted, and, as a result, data from different National Forest
131 Inventories adopting different definitions cannot be directly aggregated. Chirici et al. (2011)
132 report that some of the most used definitions for stand age are: the mean age of the trees in
133 the upper (dominant) tree layer, the mean tree age of the dominant species (in the upper
134 layer), or the mean age of all trees weighted with basal area or crown cover.

135 For these reasons, we think that a method for deriving a spatial estimation of forest stand age
136 without directly using the forest age information acquired by the NFIs in the field would be
137 useful, especially within a pan-European context when the information from different NFIs
138 plots would have to be merged has to be merged.

139 In this study, we propose a simple approach based on the integration of remotely sensed data
140 and inverted yield models. We tested the method to even aged forests located in a study area
141 coincident with the administrative region of Molise in central Italy, following three sequential
142 steps. First, we produced a high resolution growing stock volume map by integrating optical
143 remotely sensed imagery from IRS LISS-III and field data from a local forest inventory,
144 using the well-known k-NN method. Second, for each one of the even-aged forest categories
145 in the study area, we developed inverted yield equations in order to be able to predict forest
146 stand age as a function of the growing stock volume. Third the forest age map was obtained
147 by applying forest-category-specific inverted yield equations to the growing stock volume
148 map, and its accuracy was evaluated using an independent information available in local
149 standwise forest inventories created for supporting forest management at a local level.

150 **Materials**

151 *Study area and input data*

152 The study area, coincident with the administrative region of Molise in central Italy, covers
153 approximately 443758 ha (Fig. 1) and is characterized by remarkable environmental
154 heterogeneity with altitudes ranging from sea level (Adriatic coast) in the east to 2050 m a.s.l.
155 at the Matese massif on the south. The climate in this region varies from Mediterranean to
156 Temperate (Anonymous 2002).

157 [Fig. 1 should be here]

158 Forests and other wooded lands cover 35% of the Molise region and they are dominated by
159 deciduous broad-leaved formations (Garfi & Marchetti 2011). Turkey oak forests (*Quercus*
160 *cerris*) represent the most common forests in this region (with the 40% of the total forest
161 area). In some hilly warm sectors, downy oaks (*Quercus pubescens*) dominate the forest
162 landscape (22% of forests). In the mountain sectors (above 1200 m a.s.l.) and at the colder
163 sites, deciduous oak forests are replaced by beech forests (*Fagus sylvatica*) (9.5% of the
164 forest area). Other forest categories which are less represented include the hop-hornbeam
165 forests (*Ostrya carpinifolia*), holm oak forests (*Quercus ilex*) and chestnut forests (*Castanea*
166 *sativa*). The autochthonous silver-fir forest (*Abies alba*, 0.3% of the forest area) is of
167 particular interest because it represents a post-glacial relict that only survives in small areas
168 of the Apennines (Lombardi et al. 2012, Santopuoli et al. 2014). Most of the forests in this
169 region are even-aged, and are mainly managed as coppice for the production of firewood. In
170 unmanaged areas, open formations and irregular structured forests (e.g., invasive broad-
171 leaved forests and riparian forests) are also present (Garfi & Marchetti 2011).

172 In this study we used cloud free IRS LISS III imagery acquired in the summer of 2006 (Fig.
173 1) for predicting the growing stock volume. The dataset is made of four bands acquired
174 between the green and near infrared wavelengths (0.52-0.59 μm , 0.62-0.68 μm , 0.77-0.86
175 μm , 1.55-1.70 μm) with a nominal spatial resolution of 23.5 meters (resampled at 20 m). The

176 image is part of the IMAGE2006 dataset. See Müller et al. (2006) for a more detailed
177 description.

178 A map of the even-aged forest categories in the region was obtained from a fine scale
179 (1:10,000) map of forest types (Garfi & Marchetti 2011). We selected the following eight
180 even-aged categories: holm oak forests, downy oak forests, hop-hornbeam forests, chestnut
181 forests, turkey oak forests, beech forests, coniferous plantations (coastal/plain coniferous
182 plantations and mountainous/sub-mountainous coniferous plantations) and silver-fir forests.
183 The map of even-aged forest categories, which covers 128402 ha represents approximately
184 the 80% of the total forest area in the region (Fig. 2).

185 [Fig. 2 should be here]

186 Geocoded field data regarding the growing stock volume was collected in the framework of a
187 regional Forest Inventory that was carried out in 2006. The Forest Inventory was conducted
188 using a standard two-phase tessellated random-stratified sampling (TSS) design (see Cochran
189 (1997) and Fattorini (2003) for details). All tree stems with a diameter greater than 3 cm at
190 breast height (DBH) were callipered in 304 circular plots having a radius of 13 m. In
191 addition, tree height was measured for a subsample of these trees and then estimated for the
192 rest of the trees. For each sampling unit, the growing stock volume was calculated using
193 allometric equations which were based on stem DBH and height (Castellani et al. 1984).

194 *Inverted yield models*

195 Yield refers to the final dimensions of a forest variable (e.g., growing stock volume or annual
196 increment) at the end of a certain period (Vanclay 1994). In even-aged stands, yield equations
197 predict the growing stock volume at a specified age:

$$Gs = f(age) \quad (1)$$

198 As a consequence, an inverted yield equation can be used to estimate the forest stand age as a
199 function of growing stock:

$$age = f(Gs) \quad (2)$$

200 Here, for each even-aged forest category, we selected a yield equation specifically developed
201 for the Molise region or, when this was not available, we selected an equation build up for
202 different areas in central Italy having forest stand characteristics similar to the characteristics
203 fount in the study area. Next, we inverted the yield equations. A full description of the
204 equations used in this study is reported in supplementary material 1.

205 *Local standwise forest inventories*

206 To assess the accuracy of the forest growing stock volume and stand age spatial predictions,
207 we used an independent dataset containing information about the growing stock and stand
208 age. To do so, we created a geodatabase aggregating 21 local standwise forest inventories
209 which were available in the study area. The information in these inventories is available for
210 each forest stand (Fig. 1). The growing stock volume in the forest management units was
211 calculated on the basis of specific sample plots or on the basis of full callipering. Stand age
212 was instead calculated as the number of years from the last disturbance (in most of the cases
213 the last harvesting event). This information was considered free of errors for the subsequent
214 steps of our work.

215 The information recorded in the plans refers to the year of plan preparation. We updated the
216 growing stock volumes to 2006 (the same of satellite images), using the mean annual
217 increments derived from the yield models. We also updated the forest age of each forest unit,
218 adding the number of years between the year of preparation of the inventory and the year
219 2006. Finally, we excluded all those management units with silviculture interventions
220 between the plan date and the year 2006. The growing stock volume information resulted

221 available for 446 stand units, covering an area of 4,959 ha. The mean actualized growing
 222 stock volume per stand unit was 2746.24 m³ (237 m³ ha⁻¹) and varied between a minimum of
 223 7.02 m³ and a maximum of 16,670 m³ with a standard deviation of 2.792 m³. Data regarding
 224 forest stand age was available for 305 stand units that cover 3137 ha. The mean stand age
 225 referred to the year 2006 was 52 years, varying between 1 and 127 years (standard deviation
 226 of 35 years).

227 **Methods**

228 We mapped forest age by following the general framework outlined in Fig. 3, first spatially
 229 estimating the growing stock volume using the k-NN method and then applying the inverted
 230 yield models to derive the forest stand age.

231 [Fig. 3 should be here]

232 *Growing stock map*

233 The non-parametric multivariate k-Nearest Neighbor (k-NN) method was used to spatially
 234 estimate the growing stock volume by combining the data acquired at the 304 field plots of
 235 the local forest inventory with the IRS LISS III multispectral images (Chiavetta et al. 2008,
 236 Lasserre et al. 2011). We estimated the growing stock for each IRS LISS III pixel (\widehat{GS}_t) as
 237 follows. The reference pixel set (r) are those for which the IRS LISS III spectral values and
 238 the growing stock values (GS_r) observed in the field were available, while the target pixels (t)
 239 are those for which the spectral values were available and the growing stock volume (denoted
 240 as the target variable GS_t) had to be estimated:

$$\widehat{GS}_t = \frac{\sum_{r=1}^k W_{t,r} GS_{rNN}}{\sum_{r=1}^k W_{t,r}} \quad (3)$$

241 Here, GS_{rNN} represents the growing stock values for the pixels located in the k units of the
 242 target pixel t and W is a weight factor which is inversely related to the distance between the

243 pixel t and the nearest r measured on the fourth dimensional IRS LISS III spectral band space
244 (Chirici et al. 2008). Here, the target set was made of the IRS LISS III pixels belonging to the
245 even-aged forest area (128,402 ha corresponding to 3,210,050 pixels), and the reference was
246 304 pixels, belonging to the field plots of the local forest inventory. The estimates were
247 calculated using the freely available “K-NN FOREST” software (Chirici et al. 2012b). We
248 tested three different distance measures implemented within the K-NN FOREST software
249 (Euclidean, Mahalanobis and Fuzzy) and with k values ranging between 1 and 10 based on
250 the averaged spectral values of a 3 x 3 pixel around the field plots (Chirici et al. 2008). We
251 used the Leave-One-Out (LOO) approach (Fazakas 1999, Chirici et al. 2012b) to test several
252 k-NN configuration finding that the most accurate estimation was obtained adopting the
253 Euclidean distance (De Maesschalck et al. 2000) with a $k=6$.

254 For a more detailed description of the k-NN algorithm and the assumptions and the
255 implications related to its use, we refer to the vast bibliography available (e.g., Chirici et al.
256 2008, Baffetta et al. 2009, McRoberts 2009).

257 *Forest age map*

258 The forest age map was derived on the basis of the growing stock volume map, the map of
259 forest types and the respective inverted yield equations. For each pixel which belonged to one
260 of the eight considered even-aged forest categories, we calculated the forest age as a function
261 of growing stock volume by applying the specific inverted yield equations reported in
262 supplementary material 1.

263 *Accuracy assessment*

264 To test the accuracy of the produced maps (firstly growing stock volume and secondly the
265 stand age) the per-pixel estimated values were averaged for each one of the stand units from

266 the standwise forest inventories (446 units for the growing stock volume and 305 units for the
267 stand age). Next, the per units estimated average values were compared against the field-
268 recorded data by calculating the Pearson's correlation index and the Root Mean Squared
269 Errors (RMSE) both in absolute and relative terms.

270 **Results**

271 The growing stock volume was accurately estimated through the k-NN process, the Pearson's
272 correlation coefficient between estimates and field recorded values per management unit was
273 0.979 with a RMSE of 22% (Fig. 4a). The estimated mean volume per management unit was
274 2715 m³ and varied between 0.001 and 16724 m³ with a standard deviation of 2855 m³.

275 [Fig. 4 should be here]

276 The forest age map (Fig. 5) resulted accurate (Fig. 4b) also with a correlation between
277 estimated and observed age (per forest management unit) of 0.918 and a RMSE of 15.78
278 years (30% of the real values).

279 [Fig. 5 should be here]

280 The estimated forest age per-pixel varied between 1 and 200 years with a mean of 33 years
281 (± 24 S.D.) (Fig. 6).

282 Beech and autochthonous silver-fir forests resulted as the oldest formations with average ages
283 of 56 (± 24 S.D.) and 46 (± 10 S.D.) years, respectively. In contrast, chestnut forests (15 ± 8
284 years) resulted as the youngest forests. The downy oak forests and coniferous plantations
285 were relatively young with 19 (± 21 S.D.) and 20 (± 9 S.D.) years, respectively. The remaining
286 deciduous broadleaved forests (Holm and Turkey oaks, and Hop-hornbeam forests) have
287 mean ages which were similar to the entire forested area (35 ± 20 S.D.; 37 ± 23 S.D.; 28 ± 18
288 S.D. respectively).

289 [Fig. 6 should be here]

290 After subdividing the forest age map in ten-year classes, we found that 34% of the
291 investigated forests are less than 20 years old, 60% of the forests are between 20 and 80 years
292 old and only the 6% of the forests are older than 80 years (Fig. 7). Only 0.56% of the forests
293 are older than 120 years and only 0.29% are more than 140 years. The oldest age class (190-
294 200) is rare, with a percentage of 0.02%. The dominant age classes are 10-20 and 20-30 and
295 they represent approximately 43% of the investigated forested area. Some forests are mainly
296 distributed in young formations within the region. For example, the majority of the
297 coniferous plantations are younger than 10 years, while the downy oak forests and chestnut
298 forests are dominated by stands of 10 to 20 years. Turkey oak, hop-hornbeam and holm oak
299 forests are more abundant in the young-middle-aged classes (between 10 and 40 years) and
300 are less represented in older stands. For the turkey oak and hop-hornbeam forests, the age
301 class of 20-30 years is dominant. However, for the holm oak forests, the age class of 30 - 40
302 years is dominant. Beech forests follow a bell-shaped distribution with the majority of forests
303 occurring in the age group of 30-70 years with a maximum age group of 60-70 years. In
304 contrast, although the age distribution of the silver fir forest presented some young stands, the
305 majority of these forests were between 50 and 60 years old.

306 [Fig. 7 should be here]

307 **Discussion**

308 The relatively young age of the mapped forests (43% of the forest area in our study ranges
309 between 10 and 30 years) reflects the management practices in the region where the most
310 common silvicultural system for broadleaves is coppice, with short rotation periods (usually
311 less than 25 years) (Ciancio et al. 2006). This type of management system is widespread over
312 the Italian peninsula and particularly along the Apennine landscapes, accounting for

313 approximately 3.9 million of hectares, almost the 40% of the total forest area in Italy (INFC,
314 2005). Nevertheless, approximately 0.5% of the forests in the study region are older than 140
315 years (mainly beech forests and turkey oak forests). Although this percentage is lower than
316 the percentage reported for Central European forests (Forest Europe 2011), it will likely
317 increase in the near future, mainly as a result of the abandonment of traditional harvesting
318 and silvicultural practices which was progressively registered during the industrial
319 development after the end of the II WW. A gradual increase in forest age (Vilén et al. 2012),
320 cover (Gold et al. 2006, Frate & Carranza 2013), and growing stock volume (Spiecker et al.
321 1996) was observed in Europe already.

322 The oldest formations in the study area are beech forests which are mainly managed with the
323 shelterwood system, and only marginally as coppices with longer rotation periods (30-40
324 years) when compared to other broadleaves. These areas are mainly located in the less
325 accessible upper mountain belt, where, as a result of depopulation and socio-economic
326 changes, traditional silvicultural activities were abandoned. Consequently, these older beech
327 forests are undergoing natural evolution (Ciancio et al. 2006, Carranza et al. 2012, Maesano
328 et al. 2014). Furthermore, the conversion of coppices into high forests has been implemented
329 in the last few decades in hilly and mountainous Mediterranean beech forests to attenuate the
330 negative effects of frequent clearcuttings on the soil, landscape and biodiversity (Ciancio et
331 al. 2006, Nocentini 2009). This also contribute in augmenting the forest age.

332 Oak forests resulted as relatively younger because most of them are still managed for fuel
333 wood production through the traditional coppice system: clearcut with a short rotation period
334 of 18-25 years (Ciancio & Nocentini, 2004). However, turkey oak forests tend to be older
335 than downy oak forests in the study area. Turkey oak is, in fact, typical of more hilly and sub-
336 mountain areas where many coppice forests lost their economic importance and were
337 abandoned to natural evolution or were converted to high forests (Nocentini 2009). These are

338 less accessible stands which are now ageing and resulted older than their traditional rotation
339 period (more than 25 years). Downy oak forests are instead located in the hilly sector of the
340 Mediterranean bioclimatic zone and they are more easily accessible and the anthropic
341 exploitation is more intense (Carranza et al. 2003, Acosta et al. 2005): these forests are
342 regularly clear-cut, and, as a consequence, old stands are not present.

343 Holm oak forests and hop-hornbeam forests which were traditionally grazed or coppiced for
344 coal and fuel wood production (Carranza et al. 2003) are currently present in areas with
345 young and middle aged stands. As in the cases of oak forests, young stands are located in
346 more accessible areas, while older stands are typically in less accessible and remote areas.

347 Young and small coniferous plantations are widespread in the Molise region and are
348 dominated by *Pinus nigra* and *Pinus pinaster*. Coniferous plantations were introduced in the
349 region in the 1960s, mainly for protecting against soil erosion (Anonymous 2002). The stand
350 age map probably tends to underestimated the real age of these coniferous forests because
351 they were planted on very poor and rocky soils in the Molise region. As a consequence *Pinus*
352 is growing slower and in these stands the amount of volume occurring is now lower than
353 what was expected by the application of the yield model adopted in this study. Chestnut
354 forests have a very limited presence in the investigated area (less than 360 ha), and they are
355 generally very young (between 10 and 20 years). In the region, chestnut is mainly managed
356 as coppice (frequently with short rotation periods of less than 20 years) for wood pole
357 production (Garfi & Marchetti, 2011). Finally, autochthonous silver-fir forests, although they
358 have a very limited extension in the region (less than 350 ha), are mainly present in middle-
359 aged classes, revealing their past management history based on clear-cuttings. Nowadays, the
360 traditional forest management of these pure *Abies alba* stands is abandoned for the lack of
361 economic interest in their wood assortments (Santopuoli et al. 2014) and for their high

362 conservation value (Maesano et al. 2014). These formations are now evolving naturally and
363 they will likely become older in the future.

364 **Conclusion**

365 In this study we implemented a simple and straightforward method for spatially estimating
366 forest age through the application of inverted yield models to a growing stock volume map
367 created by integrating field data and remotely sensed images through the k-NN method. For
368 even-aged forest categories in a study area of 128,402 ha, we produced a forest stand age map
369 with an accuracy (RMSE of 30%) which is in line with previous experiences developed for
370 mapping forest variables (McRoberts et al. 2010). The map has a geometric resolution of 20
371 m which is fine enough to directly support local forest management planning activities.

372 The method we propose does not use stand age measurements acquired in the field, in order
373 to avoid the inconsistencies which are frequently reported for this data (Pan et al. 2010).

374 Stand age is instead calculated on the basis of growing stock volume which is a fairly well
375 standardized variable across different countries, or, at least, it can be easily harmonized
376 (McRoberts et al. 2009). As a consequence, the method we propose can be used for
377 aggregating plot level information from NFIs of different countries, avoiding the problem of
378 merging stand age information which refer to different definitions (Chirici et al. 2011).

379 The method we propose enlarges the range of applicability of yield models (Vancaly 1994),
380 underlining the need for the development of site-specific equations for almost the main forest
381 categories covering all the main biogeographical regions of Europe (Vuokila 1965, Peng
382 2000). In the future, this method could be enhanced by considering more specific yield
383 models, including different soil fertility conditions. One of the main limitations of our
384 approach is that it can only be used to predict the age of even-aged forests, which, in any
385 case, dominate the forest area in the study area (80% of the forest area) as well as in Europe.

386 On the basis of the data available for 18 European countries (including the Russian
387 Federation) published in the last “State of European forests” (Forest Europe 2011), even-aged
388 forests represent in fact the vast majority of European forests (almost the 90% with more than
389 9 million of hectares). Thus, the method for this reason has important potential relevance at
390 pan-European level.

391 A stand age high resolution map may have different uses. Firstly, such information can
392 provide a sound basis for orienting forest management strategies, such as: facilitating a
393 general assessment of harvesting potential, identifying suitable stands to be promoted to
394 natural evolution, or contributing to determining the optimal forest management strategies
395 and maximizing the productivity of different ecosystem services.

396 Secondly, a forest age map provides important quantitative information supporting the
397 assessment of carbon sequestration of forest ecosystems and their role in bio-geo-chemical
398 cycles (He et al. 2012). For example, younger stands are expected to exhibit a rapid increase
399 in Net Primary Productivity (NPP), but older stands are expected to exhibit a slow decline. In
400 this case, the availability of forest age spatial distribution patterns could be useful for
401 assessing potential changes in the NPP under different management scenarios. Thus, potential
402 changes in important ecosystems services which are directly associated with NPP, such as
403 carbon sequestration, water cycling and regulation, soil fertility, local climate and air quality,
404 could be assessed (Zurlini et al. 2013). Stand age distribution patterns could provide a
405 relevant information source for monitoring biodiversity and recreation conditions
406 (Burrascano et al. 2013) which are generally more favorable in old and even-aged forests.
407 Finally, the proposed method could be a useful tool for contributing to the implementation of
408 forest management strategies in those regions where land ownership fragmentation
409 (Pettenella et al. 2005) or the limited commercial value of timber production determine a lack
410 of local management plans, as frequently occurs in Mediterranean areas.

411 The constant technologic development of Earth Observation techniques and the availability
412 of forest inventories may probably lead to a more consuetudinary derivation of forest
413 variables maps in the near future (McRoberts & Tomppo 2007). The procedure we tested in
414 this study may be applied to European forests at continental scales on the basis of the
415 information already available, for example on the basis of the growing stock volume map
416 from Gallaun et al. (2010), and the forest species map from Brus et al. (2012). This is
417 particularly important because forest conservation across Europe requires a common
418 approach to define and map forest age. Therefore, we hope that further studies may be
419 conducted in the future to properly test and refine this procedure and provide integrated
420 information for increasingly larger areas.

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637

638 **Figure legends**

639 **Fig. 1** Study area. The Molise region according to IRS LISS III imagery (RGB 432 color
640 composite). The locations of the forest management plans are presented in yellow.

641 **Fig. 2** Map of the even-aged forest categories which were extracted from the Forest Types
642 map. In addition, the hexagonal systematic grid used for the Regional Forest Inventory is
643 represented. The black dots represent the locations of the 304 sampling units which were
644 used for estimating the growing stock volume.

645 **Fig. 3** Flowchart representing the different steps of the proposed procedure for mapping
646 forest age.

647 **Fig. 4** Correlation between the predicted and observed total growing stock volume (a) and
648 average age (b). The bold lines represent $y=x$. The dashed lines indicate the 95% confidence
649 intervals.

650 **Fig. 5** Forest age distribution in the Molise region which was obtained as described in Fig. 3.

651 **Fig. 6** Mean estimated forest age (white circle) for the eight even-aged forest types with their
652 respective standard deviations (whiskers). The continuous horizontal line refers to the
653 average age of the forests in the region, while the dashed lines represent the regional standard
654 errors. The grey bars represent the area (in hectares) which is covered by each forest type.

655 **Fig. 7** Forest cover extension (number of pixels) per age classes (10-year intervals).

656

657 **Supplementary material**

658 **File name:** Suppl_1.docx

659 **File format:** Microsoft Word

660 **Title of data:** Supplementary material 1

661 **Description of data:** Inverted yield equations used for forest age estimation; x = age and y =
662 growing stock. In addition, references are provided for the yield models (for a complete
663 collection see Castellani 1972).

Fig. 1

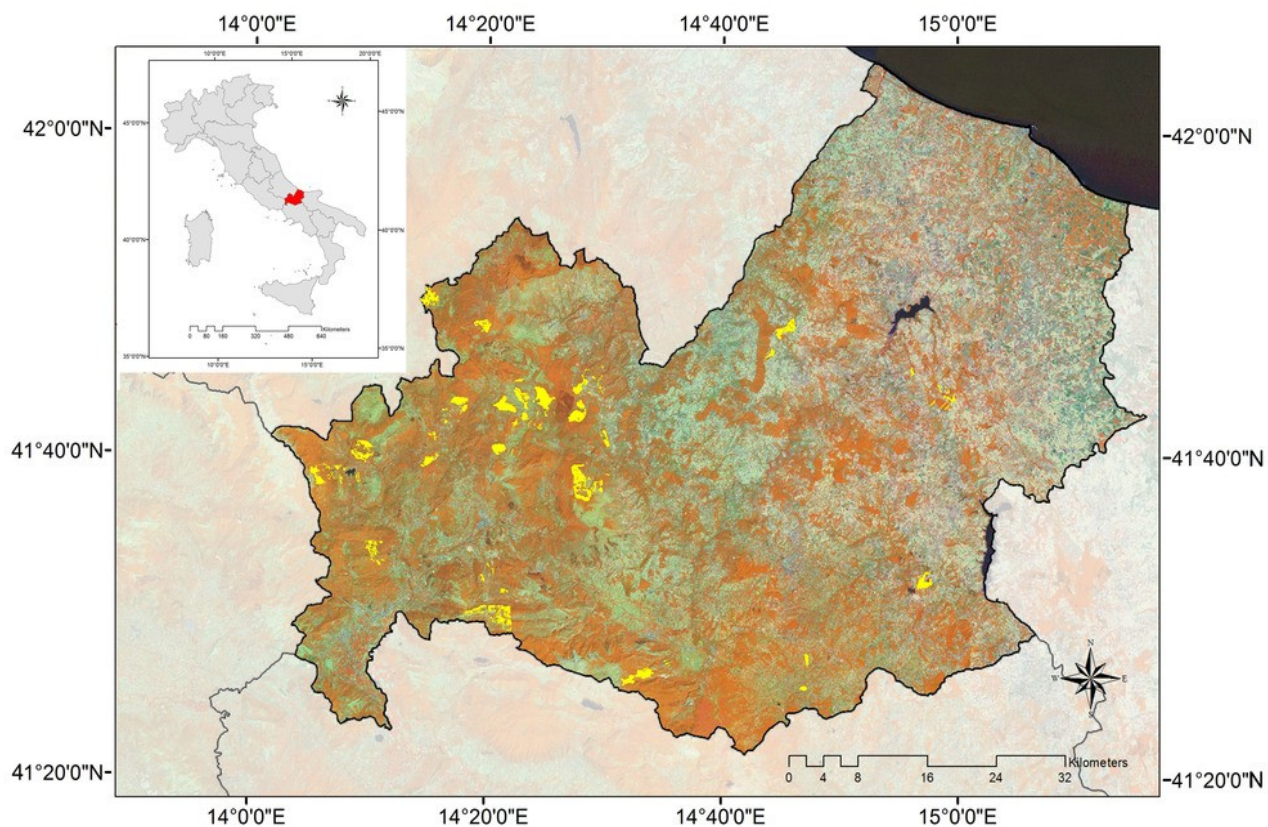


Fig. 2

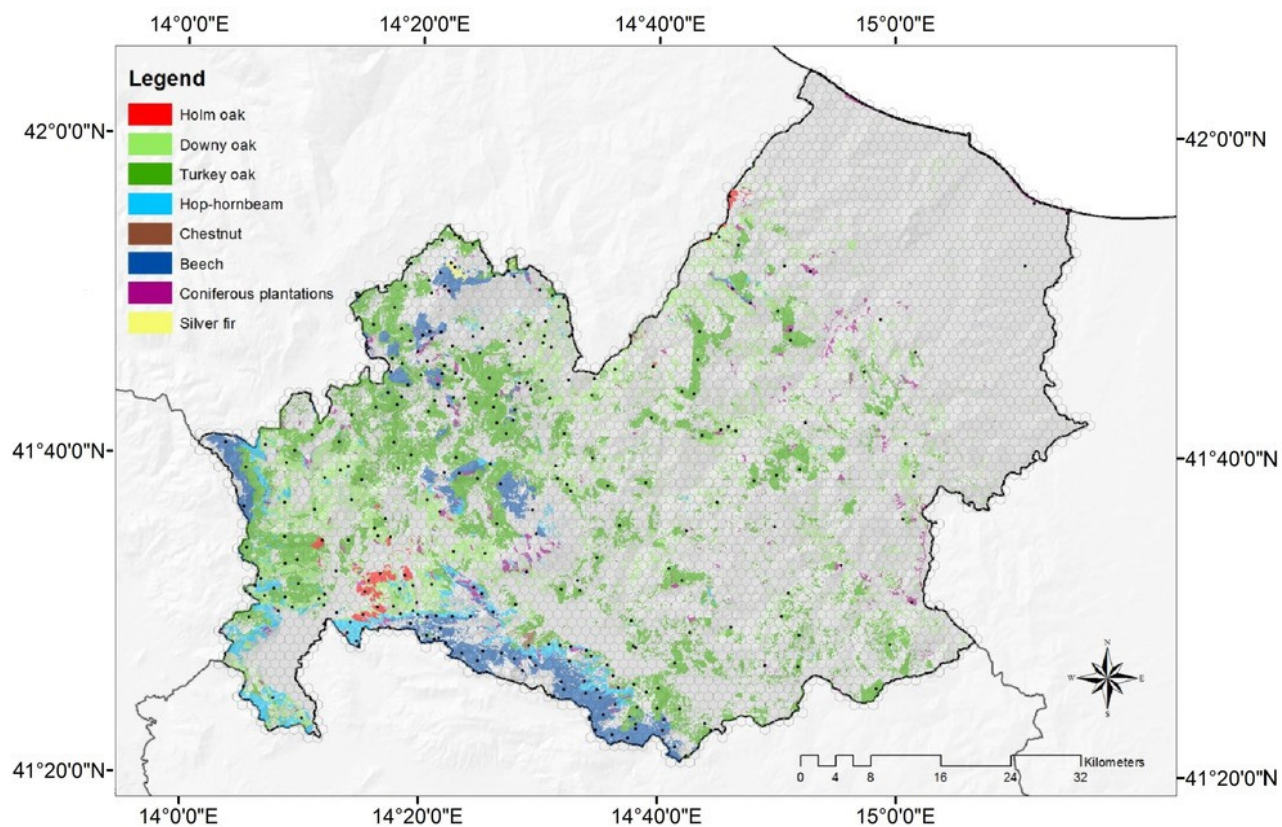


Fig. 3

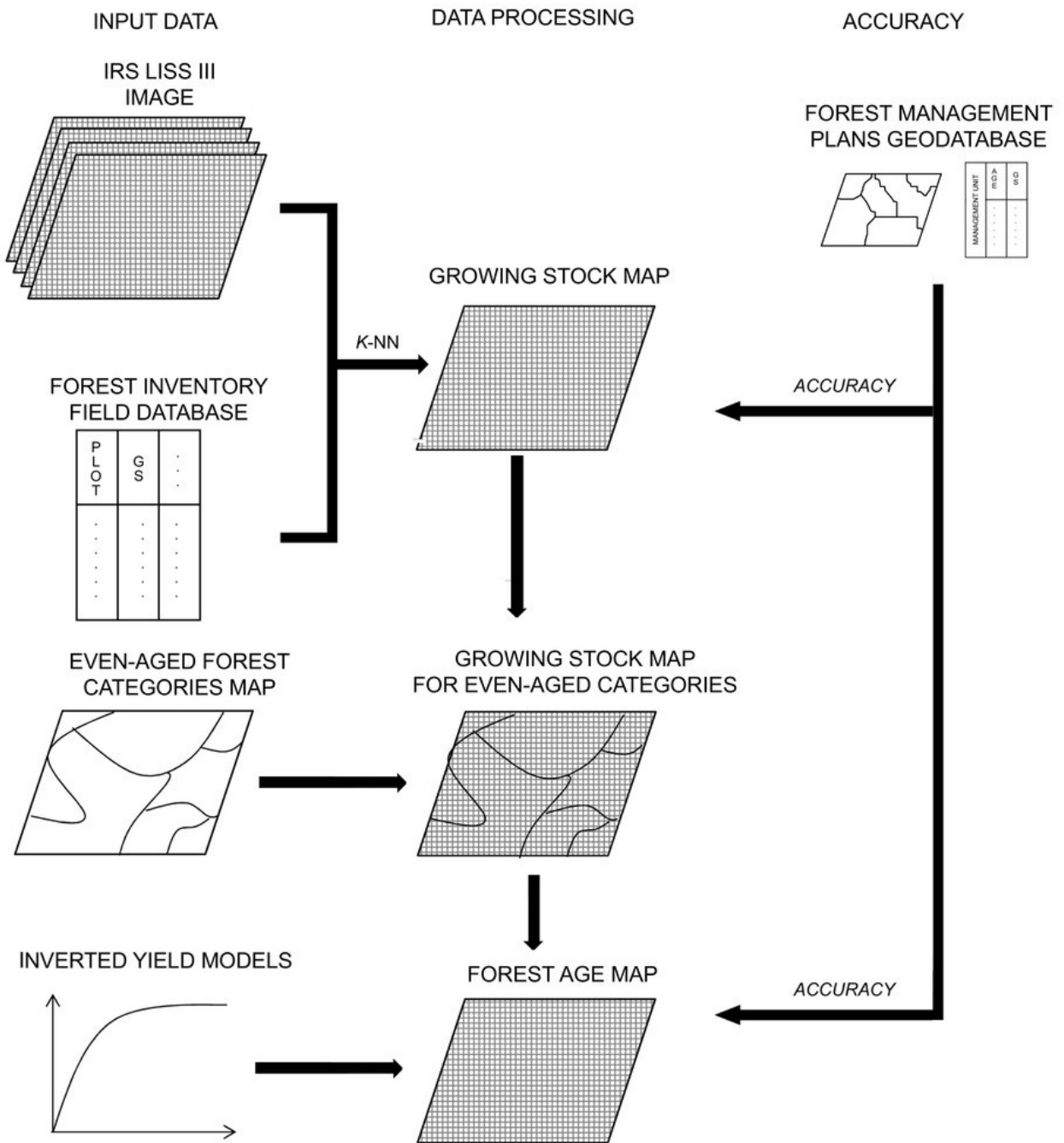


Fig. 4

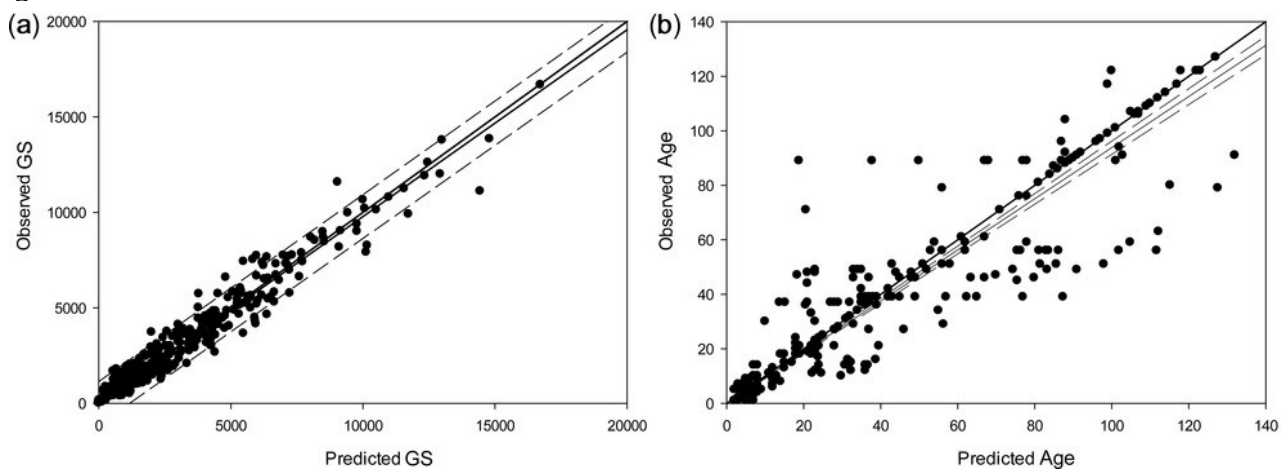


Fig. 5

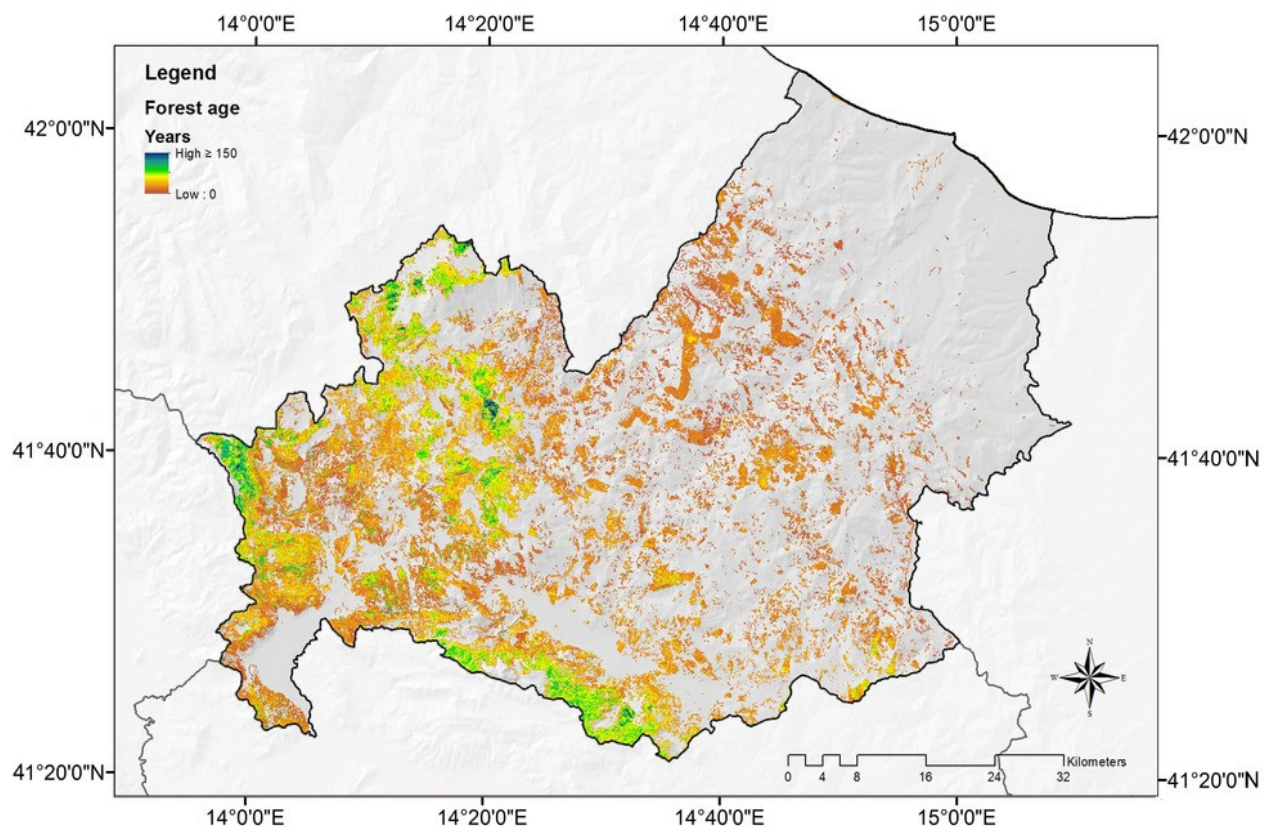


Fig. 6

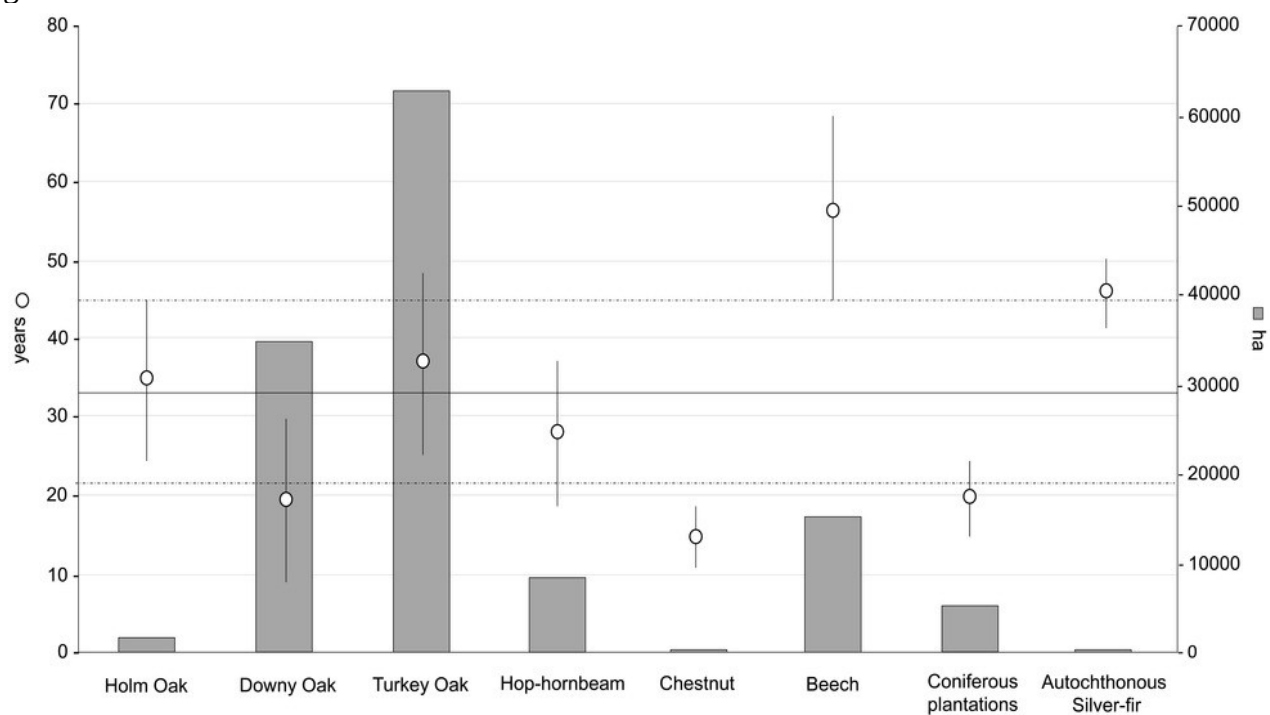
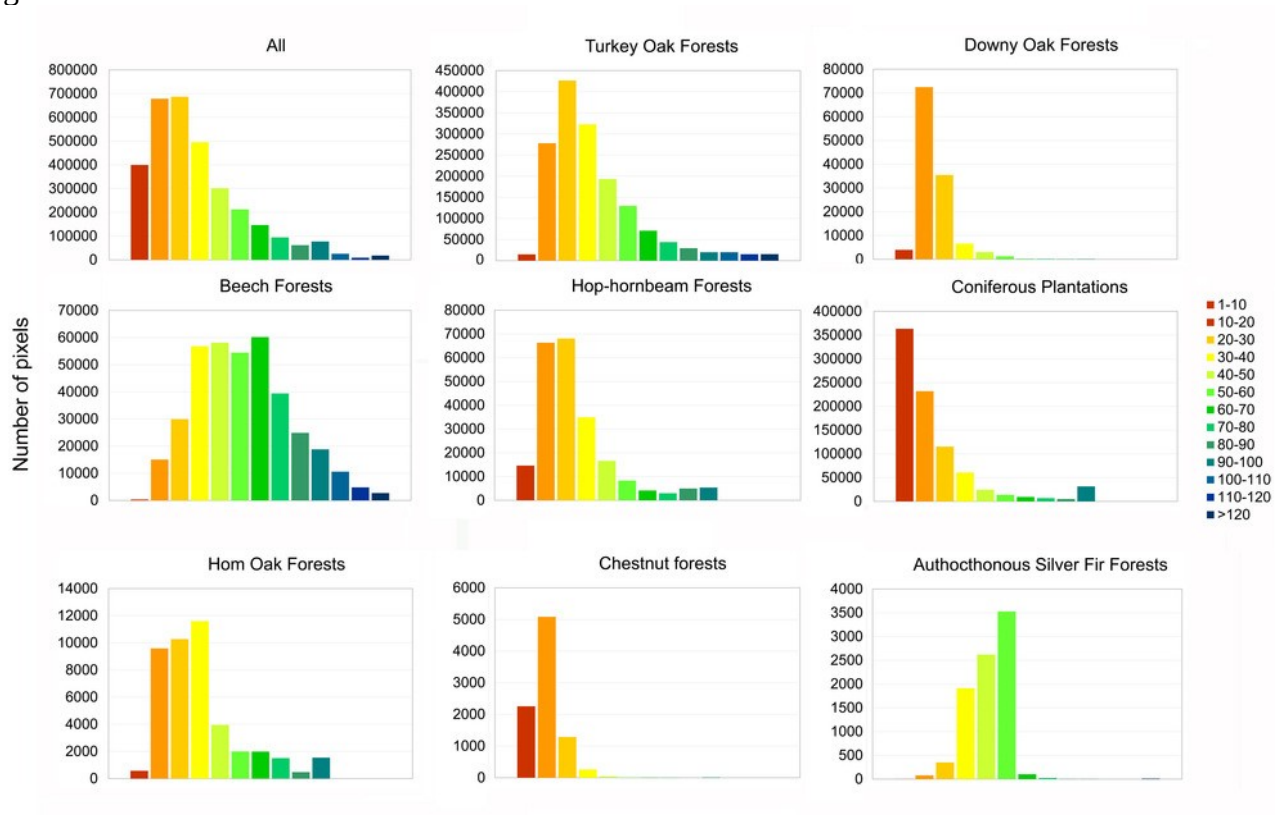


Fig. 7



Supplementary Material 1

Inverted yield equations used for forest age estimation; x = age and y = growing stock. In addition, references are provided for the yield models (for a complete collection see Castellani 1972).

Forest Category	Inverted Yield Equations	Original Yield Table
Holm Oak forests	$x = \sqrt[3]{\left(\frac{0.975371}{\frac{1}{y} - 0.00321}\right)^2}$	Poda A. Leccio del comune di Terni. Piano di assestamento per il decennio 1963-1972.
Downy oak forests	$x = \left(\frac{-5.08666}{\ln y - 5.768849}\right)^2$	Meschini A, Calliari P. Cerro, Roverella ed <i>altre specie</i> . Piano di assestamento per il decennio 1961-1970.
Hop-hornbeam forests	$x = \sqrt[3]{\left(\frac{0.741867}{\frac{1}{y} - 0.001789}\right)^2}$	Poda A. Carpino nero, Cerro e Roverella del comune di Stroncone. Piano di assestamento per il decennio 1962-1971.
Chestnut forests	$x = \left(\frac{-5.08666}{\ln y - 5.768849}\right)^2$	Castellani C (1963). Castagno dei Monti Cimini. Ricerche dendrometriche e auxometriche su fustaie di pino e faggio e sui cedui di castagno della Calabria.
Turkey Oak forests	$x = e^{\sqrt{\frac{y-2.1582}{0.821153}}}$	Patrone G (1971). Cerro del Molise. L'Italia Forestale e Montana 2:54. Gualdi V (1967). Ricerche dendrometriche ed auxometriche sui boschi cedui del Sannio. L'Italia Forestale e Montana 22: 39-51.
Beech forests	$x = \sqrt{\frac{5.434074}{\frac{1}{y} - 0.001471}}$	Patrone G (1971). Faggio del Molise. L'Italia Forestale e Montana 2: 43. Di Tella G (1944). Faggio dell'Appennino. Manuale dell'Agronomo.
Coastal/plain coniferous plantations	$x = \sqrt{\frac{-3.699746 * y}{0.002541 * y - 1}}$	Baroni A (1973). Pino domestico dei tomboli di Cecina. L'Italia Forestale e Montana, p. 191.
Mountainous/Sub-mountainous coniferous plantations	$x = \sqrt{\frac{-2.883792 * y}{0.001026 * y - 1}}$	Bernetti G, Cantiani M, Hellrigl B (1969). Pino nero e laricio. L'Italia Forestale e Montana

Autochthonous Silver- Fir forests	$x = 49.79392 - 15.03409$ $* \ln \left(\frac{y - 1072.041 + 27.6123}{-27.6123 - y} \right)$	Bernetti G, Cantiani M (1962) Abete bianco della Toscana. Accademia Italiana di Scienze Forestali
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