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Spatio-temporal functional variation of the sub-Mediterranean mountain grasslands
Variazione funzionale nello spazio e nel tempo dei prati montani sub-Mediterranei

Alessandro Bricca

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Tutor: Dott. Maurizio Cutini

Co-Tutor: Prof. Andrea Catorci

A tutte le persone che
lo hanno reso possibile

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Riassunto

Negli ultimi vent'anni il numero di studi sull'uso dei caratteri morfo-funzionali delle piante (*functional traits*) è aumentato esponenzialmente. La grande maggioranza, tuttavia, si è incentrata solo sulla misurazione di tre caratteri "facili" (*soft traits*), sostenendo tale scelta affermando che questi siano in grado di cogliere le principali *performances* della pianta:

- 1) l'altezza vegetativa (cioè, la distanza ortogonale tra il suolo e la parte più alta dell'apparato fotosintetico più alto), legata all'aspetto della competizione;
- 2) l'area fogliare specifica (cioè la differenza tra superficie fogliare fresco e peso fogliare secco), legata all'aspetto dell'acquisizione di risorse tramite fotosintesi;
- 3) il peso del seme (cioè, il peso secco del seme), legato all'aspetto della riproduzione.

Studi a livello di comunità hanno evidenziato come diversi processi ecologici sono responsabili della coesistenza delle specie. Da una parte, le condizioni ambientali filtrano le specie, selezionando solo quelle con adattamenti in grado di sopravvivere in quel particolare ambiente (*habitat filtering*). Al contrario, le interazioni biotiche, come la competizione, limitano il grado di similarità tra le specie (*limiting similarity*).

Tuttavia, le interazioni biotiche possono agire anche da filtro, rimuovendo dalla comunità quelle specie "deboli" che non siano in grado di competere con specie "forti" (*weaker competitor exclusion*), portando a *pattern* simili dell'effetto del filtraggio ambientale (*habitat filtering*). Allo stesso modo, specie funzionalmente diverse possono occupare micro-habitat, per cui l'eterogeneità ambientale (*environmental heterogeneity*) può portare a risultati funzionali simili a quelli del limite di somiglianza (*limiting similarity*). La mancanza di una metodologia che sia in grado di discernere il processo che risiede dietro il *pattern* osservato, rappresenta un fattore limitante per giungere ad una completa comprensione delle modalità di coesistenza.

La presenza di un protocollo standardizzato per la misurazione di questi caratteri ha permesso la creazione di un database globale e lo studio di *pattern* funzionali a scale maggiori. Tuttavia le informazioni ecologiche ottenute sono limitate dal momento che la componente intraspecifica è largamente trascurata, per cui la conoscenza delle relazioni tra piante e ambiente necessita di un maggiore approfondimento. Inoltre, dal momento che i tre caratteri sopra menzionati catturano solo gli aspetti della dimensione funzionale epigea delle piante, occorre rivolgere la nostra attenzione anche verso la dimensione funzionale ipogea. Per esempio, che ruolo abbiano i caratteri morfo-funzionali ipogei nel plasmare le comunità e che relazione abbiano con quelli epigei, è ancora oggetto di dibattito.

Infine, quasi la totalità degli studi funzionali hanno previsto l'uso di gradienti spaziali, i quali, se da una parte forniscono informazioni sulle relazioni pianta-ambiente, non sono in grado di cogliere correttamente carattere dinamico della vegetazione. Nello studio del dinamismo della vegetazione, risulta essere fondamentale non solo monitorare la variazione della component specifica, ma anche quella funzionale. Infatti, variazioni nelle specie non necessariamente portano a variazioni funzionali, dal momento che le specie possono mostrare *traits* simili ("ridondanza funzionale"). Monitorare l'andamento combinato dei *traits* e delle specie, cioè la sua ridondanza funzionale, rappresenta il migliore approccio per poter analizzare l'effetto che le variazioni vegetazionali hanno sul funzionamento ecosistemico.

Il mio dottorato nasce con l'intento di fornire contribute in diversi campi dell'ecologia funzionale che risultano ancora essere poco investigati. In tal caso, propongo un approccio metodologico per poter identificare il processo responsabile del *pattern* funzionale osservato; l'importanza di misurare i caratteri nei contesti ambientali in cui si studiano; investigare il ruolo che i *traits* ipogei hanno nel plasmare le comunità e che relazione sussistono con quelli epigei; fornire un primo monitoraggio che combina specie e *traits*.

In primo luogo ho studiato gli aspetti funzionali delle comunità prative del monte Velino (Appennino centrale; Abruzzo), testando l'ipotesi secondo la quale l'uso combinato di alcuni indici funzionali di comunità, possano identificare il processo responsabile del *pattern* funzionale osservato. Inoltre, lo studio prevedeva di esaminare

la relazione *traits*-altitudine, confrontandola con i risultati di studi condotti in ambiente temperato. In particolare, ho condotto un campionamento floristico su 45 plot (2x2 m) dislocati lungo un gradiente altitudinale da 1325 m fino a 2375 m. A seguito della raccolta del dato floristico, ho proceduto alla misurazione dei caratteri morfo-funzionali (altezza, area fogliare specifica e peso del seme), dividendo il gradiente altitudinale in 4 fasce misurando le specie più abbondanti per ogni fascia. Successivamente, tramite l'uso di regressioni ho analizzato la variazione, in relazione all'altitudine, di due indici funzionali che quantificano la media e la dispersione dei valori intorno la media: il CWM (*community-weighted-mean*) e MPD (*mean pairwise distance*). Soprattutto, ho testato la relazione tra i due indici, lungo il gradiente per ogni carattere. I risultati hanno evidenziato in primo luogo una relazione di tipo unimodale degli indici funzionali con l'altitudine (tranne che per il cwm dell'area fogliare specifica), che si distingue dalle relazioni lineari in ambienti temperati. In secondo luogo, investigare le relazioni tra gli indici lungo il gradiente, potrebbe rappresentare un utile approccio per quei caratteri che sono strettamente legati agli effetti della competizione sulle altre specie, come l'altezza (competizione per la luce) e il peso del seme (competizione dei germogli).

Inoltre, durante il dottorato ho partecipato a diverse collaborazioni con altri istituti nazionali e internazionali di ricerca, approfondendo tematiche quali: 1) variabilità intraspecifica a livello di popolazione; 2) relazione tra caratteri epigei e ipogeï, lungo vari gradienti ambientali.

1) Nel primo caso, ho collaborato attivamente a una ricerca condotta nella regione Marche, presso il parco dei Sibillini, con la Scuola di bioscienze e medicina veterinaria dell'Università di Camerino. L'obiettivo era studiare la plasticità del *Brachypodium genuense*, una specie in grado di proliferare nelle comunità prative montane in pochi anni dopo la cessazione di pratiche gestionali come pascolo e sfalcio, provocando una drastica diminuzione della biodiversità. Tramite l'uso di dati bibliografici, immagini satellitari e osservazioni in campo, è stato operato un disegno *random* stratificato che ha portato alla selezione di 221 plot (20x20 cm) in diverse condizioni ambientali. In ogni plot sono stati misurati dati micro- (edafici), meso- (topografici) e macro-ambientali (altitudine), oltre al valore di copertura del *Brachypodium genuense* e al valore di area fogliare specifica e altezza. Analisi multivariate e di partizione della varianza hanno evidenziato una grande plasticità per i caratteri misurati del *Brachypodium genuense*, supportando la descrizione qualitative data da Grime alle specie di questo genere come "competitive-stress tolleranti": strategie competitive, cioè alto tasso di acquisizione e basso tasso di accumulazione di risorse, sono state trovate in ambienti più produttivi, strategie stress tolleranti, cioè bassa acquisizione e alto accumulo di risorse, in ambienti meno produttivi. La capacità che questa specie, *Brachypodium genuense*, mostra nel diffondersi in diverse condizioni ambientali, dominando le varie comunità, sembra dovuta quindi non solo dalla presenza di strategie clonali, ma anche a questa grande plasticità nel modulare la propria *performances* a seguito al variare delle condizioni ambientali.

2) Nel secondo caso, ho collaborato con l'istituto di botanica dell'Accademia delle Scienze della Repubblica Ceca, con il quale ho partecipato ad uno studio condotto a scala nazionale. Utilizzando il database fitosociologico della Repubblica Ceca e quello dei caratteri funzionali globale (TRY) si è proceduto a studiare che rapporti sussistono tra i caratteri epigei (altezza, area fogliare specifica e peso del seme) e i caratteri ipogeï (sia i vari aspetti clonali, che quelli legati alle gemme) al variare delle condizioni ambientali a macroscale (livello di *habitat*). I valori di Ellemberg sono stati usati per delineare i vari gradienti ambientali e le analisi sono state condotte con i modelli nulli. I risultati evidenziano come la dominanza e diversità dei caratteri legati alla clonalità e alle gemme siano influenzate dai gradienti di luce, temperature, umidità, pH e nutrienti del suolo, molto più rispetto ai caratteri epigeï, sottolineando quindi un maggior filtro ambientale per i primi. Inoltre, abbiamo evidenziato come i trend epigeï e quelli ipogeï mostrino un *pattern* opposto.

Sempre con lo stesso istituto di botanica dell'Accademia delle Scienze della Repubblica Ceca, ho condotto uno studio su un tema simile, cioè la relazione tra caratteri epigeï e ipogeï, misurando un nuovo carattere funzionale ipogeï, che supponiamo essere legato alla capacità delle piante di conservare le risorse: variazione della quantità d'acqua dell'organo ipogeï. Sono ritornato sullo stesso gradiente altitudinale del Monte Velino (Appennino central; Abruzzo), dal momento che, utilizzando gli stessi plots e le stesse specie

selezionate l'anno precedente, ho proceduto alla misurazione di questo nuovo carattere assieme alla sua controparte epigei (variazione della quantità d'acqua nella foglia) e di poter mettere in relazione questi nuovi dati, anche ai dati misurati precedentemente (altezza, area specifica fogliare, peso del seme). Ho condotto analisi sia per testare come variano le strategie di questi due caratteri a livello di comunità con gli stessi indici funzionali di media e dispersione, poi ho analizzato la presenza o meno di coordinazione tra tutti i vari caratteri (altezza, area fogliare specifica, peso del seme, variazione della quantità d'acqua sia della foglia che dell'organo ipogeo). Infine, ho proceduto anche a quantificare quanta variabilità, in questi due nuovi *traits*, fosse dovuta a una variazione intraspecifica o interspecifica. Le analisi a livello di comunità lungo gradiente hanno evidenziato un *pattern* di diversità opposto: laddove si riducono le strategie legate alla foglia, aumentano quelle legate agli organi ipogei e viceversa. Inoltre, il contributo della variazione intraspecifica, per il carattere legato alla conservazione dell'acqua, lungo il gradiente altitudinale è maggiore di quello della variazione interspecifica, evidenziando l'importanza di non considerare il carattere "fisso" nella specie lungo gradienti ambientali. Infine, l'analisi a livello di specie mostra che il contenuto secco dell'organo ipogeo sembra cogliere un asse indipendente nello spettro delle *performances* delle piante. I risultati, in linea con i precedenti, hanno evidenziato come tra i caratteri epigei e quelli ipogei, possa intercorrere una relazione di tipo opposta e che occorrerebbe estendere le misurazioni funzionali, ai caratteri ipogei con particolare attenzione al contenuto secco dell'organo ipogeo.

Infine, l'ultimo contributo del dottorato riguarda il monitoraggio delle variazioni funzionali e delle specie nel tempo. Disponendo di due passati periodi di campionamento floristico (2006, 2011) ho eseguito lo stesso approccio menzionato nel primo contributo, per la misurazione dei *traits* lungo il gradiente altitudinale del Monte Velino: ho diviso il gradiente altitudinale secondo le stesse 4 fasce e ho misurato i caratteri (altezza, area fogliare specifica e peso del seme) per le specie che risultavano abbondanti per ogni fascia usando lo stesso numero e identità dei plots. Dal momento che le misurazioni dei caratteri mofo-funzionali del 2016 erano disponibili, ho provveduto a misurare solo i caratteri per le specie del 2006 e del 2011. In questo caso, la variabilità intraspecifica non è stata considerata. Analisi di varianza (ANOVAs) sono state condotte per esaminare la variazione temporale per ogni fascia dei caratteri funzionali a livello di comunità e le variazioni tassonomiche. I risultati hanno evidenziato come le fasce intermedie siano soggette a un maggior dinamismo nel tempo sia funzionale che tassonomico, lasciando supporre un aumento nel tempo dei servizi ecosistemici. Nella fascia bassa e alta si osserva nel tempo un solo aumento della componente specifica, lasciando intendere come per questi *traits* si sia raggiunta la massima diversità e di conseguenza, i servizi ecosistemici non variano.

L'area di studio dove ho deciso di svolgere la mia attività di ricerca è rappresentata dalle praterie montane dell'Italia Centrale (eccetto un contributo fatto in collaborazione con i colleghi della Repubblica Ceca). Questo *habitat* riveste una particolare importanza sia per l'elevata ricchezza floristica che per l'elevato numero di specie vegetale e animali di interesse conservazionistico. Inoltre, questi ambienti svolgono servizi ecosistemici di interesse comunitario e benefici economici, come pascolo e sfalcio. Tuttavia, a seguito della Seconda Guerra mondiale, cambiamenti socio-economici hanno portato all'abbandono dei prati e la migrazione verso le città sta influenzando negativamente questi *habitat*. Per tutta l'Europa, così come lungo tutto l'Appennino, le cessazioni delle pratiche agro-pastorali sta portando all'avanzamento della foresta. Investigare nella stessa area di studio vari aspetti funzionali (che relazione sussiste tra i *traits* epigei e ipogei) a varie scale biologiche (popolazione e comunità), nonché su diversi gradienti (spaziali che temporali), ritengo che possa permettere una maggiore comprensione del funzionamento di questi ecosistemi nonché del loro dinamismo, al fine di sviluppare pratiche gestionali che non ne compromettano il funzionamento, garantendo al contempo i servizi ecosistemici nonché la conservazione di specie floristiche di interesse. Inoltre, non disponendo di molte informazioni sui prati montani mediterranei, questo dottorato di ricerca si prefigge lo scopo di contribuire nell'aumentare la conoscenza di questo particolare *habitat*.

In generale, i risultati di questa tesi di dottorato evidenziano come diversi campi attualmente poco investigati dell'ecologia funzionale rivestino molta importanza. L'approccio metodologico proposto potrebbe risultare utile ma tuttavia non generalizzabile, dal momento che sembra che si presterebbe solo per alcuni *traits*, quelli legati alla capacità delle specie di sopprimere le altre (competizione asimmetrica o effetto della competizione). Le analisi tra *traits* epigei e ipogei a livello di comunità hanno aggiunto prove alla teoria secondo cui le relazioni che intercorrono tra loro si traducano in un *pattern* opposto. A livello di specie la mancata relazione tra il carattere ipogeo legato alla conservazione delle risorse e i *traits* epigei, in particolare alla controparte funzionale epigea, sembra evidenziare un nuovo asse indipendente delle strategie funzionali delle piante che contribuirebbe a spiegare come fanno le specie a coesistere.

Nelle analisi lungo gradienti, la componente intraspecifica occupa un ruolo fondamentale, per cui tralasciare questa informazione porta a una visione parziale delle relazioni che intercorrono tra pianta e ambiente.

Infine, l'uso di plots permanenti può fornire un utile approccio per poter descrivere le variazioni temporali legati agli aspetti funzionali e tassonomici delle comunità e investigare quindi, non solo quali specie e in che misura cambiano nel tempo, ma se queste variazioni si ripercuotono sul funzionamento e sui servizi ecosistemici che un dato *trait* svolge.

Complessivamente, un approccio funzionale multiplo che prevede analisi spaziali e temporali, aspetti funzionali epigei e ipogei, quantificare il contributo della variazione intraspecifica funzionale a livello di comunità e di popolazione, possa garantire una maggior comprensione dei meccanismi di coesistenza delle specie e dei loro adattamenti all'ambiente. Investigare questi aspetti nei prati montani mediterranei assume inoltre una certa rilevanza dal momento che il loro status è fortemente minacciato dall'abbandono e rappresenta il primo passo per lo sviluppo di politiche manageriali volte a mantenere intatti i servizi e il funzionamento dei suddetti *habitat*.

Papers published or prepared during the PhD

Functional ecology

Vojtko, E., Freitag, M., **Bricca, A.**, Martello, F., Compañ, J. M., Küttim, M., ... & Götzenberger, L. (2017). Clonal vs leaf-height-seed (LHS) traits: which are filtered more strongly across habitats? *Folia Geobotanica*, 52(3-4), 269-281.

Chelli, S., Marignani, M., Barni, E., Petraglia, A., Puglielli, G., Wellstein, C., ..., **Bricca, A.**, ... Cerabolini, B. E.L., (2019) Plant-environmental interactions through a functional traits perspective: a review of Italian studies. *Plant Biosystems*.

Forest functional ecology

Scolastri, A., **Bricca, A.**, Cancellieri, L., & Cutini, M. (2017). Understory functional response to different management strategies in Mediterranean beech forests (central Apennines, Italy). *Forest Ecology and Management*, 400, 665-676.

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Grassland functional ecology

Tardella, F. M., **Bricca, A.**, Piermarteri, K., Postiglione, N., & Catorci, A. (2017). Context-dependent variation of SLA and plant height of a dominant, invasive tall grass (*Brachypodium genuense*) in sub-Mediterranean grasslands. *Flora*, 229, 116-123.

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Bricca, A., Conti, L., Tardella, M.F., Catorci, A., Iocchi, M., Theurillat, J-P., Cutini, M., (2018) Is the relationship between trait mean and trait dispersion useful to reveal community assembly processes? A case study from sub-Mediterranean mountain grasslands. *Plant Ecology*. (major revision)

Bricca A., Ottaviani G., Götzenberger L., De Bello F., Cutini M., Klimešová J. Belowground organ dry matter content: contrasting patterns of trait variation at species and community level. (in prep)

Bricca, A., Cutini, M., Species composition vs functional trait variation along temporal gradient: permanent plot-based approach. (in prep).

Functional traits: an overview

Predicting plant responses to changing environments has long been a primary topic in ecology (von Humboldt & Bonpland, 1807; Schimper, 1903; Whittaker, 1960). Linking species and community responses across environmental gradients can facilitate inferences and predictions of effects caused by variations in abiotic and biotic conditions on plants (Lavorel & Garnier, 2002; Cornwell & Ackerly, 2009; Funk et al., 2017). This task can be effectively achieved by investigating the plant diversity not only with a taxonomic approach, i.e. species composition, but also focusing on species functional traits (Diaz & Cabido, 1997, 2001; Violle et al., 2007). Functional traits, defined as any measurable feature affecting individual fitness (Violle et al., 2007), are increasingly recognized as useful tools for understanding mechanisms driving species' coexistence across different environmental conditions (Lavorel & Garnier, 2002; Garnier et al., 2016). Capturing different aspects of species' resource use and habitat requirements, they may be directly linked to ecosystem functioning, providing a more mechanistic understanding of species and community response to environmental factors (Lavorel & Garnier, 2002; Lavorel et al., 2011; Mason et al., 2011; Kleyer et al., 2012), disturbance regime (de Bello et al., 2012a) and management practices (Scolastri et al., 2017), improving biodiversity predictions under environmental change (Suding et al., 2008; Laliberté et al., 2010; Reu et al., 2011). Moreover, they have helped to reveal community assembly rules, i.e. how species co-exist in an assemblage (Götzenberger et al., 2012; de Bello et al., 2013b).

Despite the increasing number of functional studies (Garnier et al., 2016; Diaz et al., 2016; Funk et al., 2017), we are still far away from having a precise scheme of functional strategies that allow the prediction of plants' distribution along environmental gradient, since relevant functional dimensions are still poorly assessed: 1) how identify which assembly process lead to the same functional pattern (de Bello et al., 2013b); 2) intraspecific trait variation is often neglected in plant-environmental relationships (Lepš et al., 2011; Violle et al., 2012); 3) a large part of plant traits' variation, for example belowground functional space, remains still unexplored (Shipley et al., 2016; Klimešová et al., 2018); 4) there is a lack knowledge regarding how functional traits and species diversity may change along temporal gradient (Wesche et al., 2012).

1) Community assembly rules

There is general consensus that plant communities are the results of different filters acting on a regional pool of species (Cornwell & Ackerly, 2009; Götzenberger et al., 2011; de Bello et al., 2013a, 2013b). According to niche theory, abiotic factors select only those species with a viable trait-combination ("habitat filtering"; Keddy, 1992) promoting the coexistence of species with similar ecological preference ("functional convergence"; Weiher & Keddy, 1995; Cornwell & Ackerly, 2009). For example, in alpine environmental, species without frost-tolerance traits will be excluded, and the resulting community will be more homogeneous

from functional point of view (Davis et al., 1999). Contrary, as the competitive exclusion principle of Gause (1964) assesses that two species are not able to coexist if their own niches are completely overlapped, negative biotic interactions are expected to constrain the overlap of ecological similarity between coexisting species (“limiting similarity”; MacArthur & Levins, 1967), and consequently the species will tend to diverge from each other (“functional divergence”).

These processes might operate within a given plant community and the dominance of one process over the other will depend on the scale at which the community is studied (de Bello et al., 2013a, 2013b), on the given trait considered (Grime, 2006) and on the intensity of the environmental conditions (Weiher & Keddy, 1995; Cornwell & Ackerly, 2009; May et al., 2013; Schellenberger-Costa et al., 2017). However, inferring processes from trait patterns has its unresolved issues; for example, negative biotic interactions of ‘weaker-competitor exclusion’ are frequently neglected. Indeed, negative biotic interactions can also promote functional convergence, since highly competitive species may filter out weaker competitors (de Bello, 2012b; Grime, 2006 b; Mayfield & Levine, 2010). Furthermore, environmental heterogeneity can lead to trait divergence since functionally different species might occupy different environmental micro-habitats within the site (de Bello et al., 2012b; Conti et al., 2017). Therefore, to shed light on the link between functional patterns and assembly processes, it may be useful to study not only the trait variation along the environmental gradient (i.e. convergence or divergence pattern), but also the relationship between different functional metrics along such gradient. Usual way how to study functional composition on community level is throughout the community weighted mean (hereafter CWM; Garnier et al., 2004) and trait dispersion, i.e. how much are functionally dissimilar co-existence species (Villegger et al., 2008; Mason et al., 2005; Laliberté and Legendre, 2010). CWM reflects the trait-environmental relationship quantifying shift in the mean trait values due to environmental selection for the given functional trait. The other component is functional dispersion (FDis) which reflect the degree of functional dissimilarity among the community and can be expressed with different metrics (Mason et al., 2005; Villegger et al., 2008; Laliberté and Legendre, 2010). Communities characterized by a higher dispersion (i.e. trait divergence) highlighted co-existence of species with a wider variety of functional strategy (i.e. niche differentiation), compared to community characterized by lower dispersion (i.e. trait convergence) in which the species that coexist are functionally similar (i.e. niche overlapping).

Even if it has been demonstrated that these metrics are mathematically correlated (Diaz et al., 2013), so far there is a lack of study that try to quantify their relationship with biological communities. We suggest that analysing the changes in the relationship between mean and dispersion of a given trait within the community, could help to reveal the process leading to the observed functional pattern. For example, a high community mean value related to low functional dispersion value for plant height (functional convergence towards high values of plant height) could be interpreted as a sign of biotic filtering (weaker competitor exclusion), since taller species might exclude shorter species (Lepš 1999; Lepš et al., 2011). On the contrary, a low community mean value related to a low functional dispersion value (functional convergence towards low values of plant height) can indicate the dominance of environmental filtering, with the environmental constraint avoiding the develop of taller species.

2) Relevance of intraspecific trait variability

In recent years the number of studies using functional traits have increased (Garnier et al., 2016; Diaz et al., 2016; Funk et al., 2017), fostered also by the accumulation of plant trait data in global database (e.g. TRY, Kattge et al., 2011). The use of mean trait values in ecological studies is justified on the assumption and some evidence that more variation occurs between than within species (e.g. Koehler et al., 2012). However, functional traits variation within species can be substantial and ecologically important (e.g. Clark, 2010; Lepš et al., 2011). Indeed, intraspecific trait variability (hereafter, ITV) may affect plant responses to abiotic and biotic factors (Fridley & Grime, 2010; Conti et al., 2018), and additionally, it can influence key ecosystem functions like productivity (Enquist et al., 2015), nutrient cycles (Madritch & Lindroth, 2015), litter decomposition (Schweitzer et al., 2012). ITV may be assessed at different level of organization: from variation within an individual plant to variation within a community (Funk et al., 2017).

Population-level studies highlight the magnitude of ITV that may be observed within a species, assessing also the degree to which is shaped by genetic variation and phenotypic plasticity, broadly defined as the capacity of an individual to alter their growth in response to disturbance and fluctuating environmental conditions (Valladares et al., 2007). These studies are also noteworthy in the light of plant invasion management (Conti et al., 2018). Indeed, invasive plant are able to spread into communities, affecting negatively the biodiversity (Catorci et al., 2011). Thus, understanding the range of their plasticity, i.e. how much are able to changes their performances at the variation of environmental conditions, represent an essential step to predict the rate of spreading under different environmental conditions.

Ultimately, ITV can affect also community composition (Lepš et al., 2011; Volf et al., 2016). Indeed, community trait variation may be caused either by species composition (e.g. species turnover and change in relative abundance, while the trait values of individuals within the species remain constant), by ITV (e.g. species composition unchanged but the trait value of the individuals within a species changed), and by the covariation between ITV and species composition (Lepš et al., 2011; Volf et al., 2016). Negative covariation, where species composition variation and ITVs effects compensate each other (e.g. species with higher given trait value are selected but are promoted individuals with opposite value of the given trait), can lead to an absence of community response. In contrast, positive covariation (e.g. species with higher given trait value are selected and within the species, individuals with higher given trait value are promoted) can result in a reinforcement in the community responses (Lepš et al. 2011; Volf et al., 2016). These effects remain largely undetected with trait retrieved from database. Thus, predicting community responses to environmental changes requires disentangling the relative contribute of species composition, ITV and their covariation (Lepš et al. 2011; Volf et al., 2016).

3) Belowground functional space

A huge body of functional ecology studies have focused on three easy-collecting traits, that is, specific leaf area (L), plant height at maturity (H) and seed mass (S), i.e. traits capturing the main aboveground functional plant strategies: competition, resource acquisition and dispersal (LHS scheme of Westoby et al., 1998; Diaz et

al., 2016; Lalibert et al., 2017). However, aboveground traits do not provide information on belowground processes and functioning (Klimešová & Herben, 2015; Kramer et al., 2016), leaving a large part of plant variation unexplored (Klimešová & Herben 2015; Shipley et al., 2016). Even if currently studies are highlighting the importance to look on belowground functional traits, this research direction is still scarce investigated (Klimešová & Herben, 2015; Klimešová et al., 2018). Belowground plant ecology research is experiencing a spiking interest and currently belowground database is available (Iversen et al., 2017), however advancements are mainly focused on traits related to fine roots and mycorrhizal associations reflecting only root acquisition strategy (Laughlin et al., 2010; Kramer-Walter et al., 2016; Laliberté et al., 2017).

Recently ecologists highlighted other belowground important traits capturing different function from resource acquisition (Shipley and Vu 2002; de Bello et al., 2012a; Klimešová & Herben, 2015; Klimešová et al., 2018), but these studies are underrepresented and the belowground functioning space, compared to aboveground counterpart, remain poorly investigated (Laliberté et al., 2017). Easy-collecting traits, capturing different function from resource acquisition are urgently needed. Moreover, as different traits usually contribute to coexistence in community differently, as was found for different aboveground traits (Grime 2006 b), we can expect that this hold especially true when we consider aboveground versus belowground traits. Indeed, previous works suggested that belowground functional traits may show opposite pattern to aboveground (Spasojevic & Suding 2012), for example, vertical growth may be subject to trait convergence, while soil foraging traits may underlie trait divergence (Mayfield & Levine, 2010)

4) The use of functional traits for long-term studies

Predicting ecosystem responses to global change, such as land use and climate, is a major challenge in ecology (McIntyre et al., 1999; Lavorel et al., 2002; Suding et al., 2008; Hoegh-Guldberg et al., 2008) and critical step in that challenge is to understand how changing environmental conditions influence processes of vegetation dynamics.

Studies on vegetation dynamics are generally based on “indirect” approach, i.e. on the comparison of sites along land-use gradients or climatic gradients (space-for-time substitution or chronosequences; Laliberté, 2010; Catorci et al., 2011; Sternberg et al., 2011). This approach typically involves extrapolation from spatially distinct sites that are expected to represent certain stages in a temporal succession, to temporal patterns (Pickett, 1989). On the contrary, the “direct” approach involves monitoring of dynamic, long-term vegetation changes (Schmidt, 1988), which necessarily involves long study periods. However, pattern of vegetation changing over time are not sufficiently understood because most studies are based on space-for-time substitution (Wesche et al., 2012). Indeed, existing environments as proxies for environments under future changed are not, in themselves, sufficient to predict changes in vegetation (Sternberg et al., 2011). Nevertheless, long-term studies on natural gradients are considered to be powerful tools for monitoring changes in biodiversity, exploring the causes beyond those changes and assessing the conservation status of particular habitats (Giarrizzo et al., 2016; Sperandii et al., 2017). Few studies involves long-term periods are concentrate mainly on variation in the species diversity (Britton et al., 2009; Pauli et al., 2012; Sperandii et al., 2017).

Generally, species richness is considered as only measure of biodiversity (Balvanera et al., 2006), asserting a positive relationship with ecosystem functioning and services (Diaz & Cabido, 2001). However, evidences about species loss affecting ecosystem functioning and services via species traits are increasing (Chapin et al., 2000; Hooper et al., 2005; Suding et al., 2008; de Bello et al., 2010; Laliberté et al., 2010). Moreover, assessing ecosystem functioning with a species composition approach may lead to misleading interpretation. Indeed, functional traits variation could not be infer from species variation, since coexistence species may show similar traits (“functional redundancy”; de Bello et al., 2009) and the decreasing in species diversity may unchange the functional traits of the community (de Bello et al., 2009; Carmona et al., 2012; Malavasi et al., 2016). Thus, the decreasing of species with similar strategy probably will not affect the ecosystem functioning and services (de Bello et al., 2009; Laliberté et al., 2010).

While the impact of global change has been mostly quantified on species loss (Pimm & Raven 2000; Pauli et al., 2012; Sperandii et al., 2017), its impacts on functional redundancy over time remain much less clear. Indeed, to date few studies has attempted to quantify only the temporal shift in community functional traits (Giarrizzo et al., 2016).

My PhD studies have been performed in the sub-mediterranean mountain semi-natural grasslands of Central Italy. Furthermore, during my PhD project, a valuable collaboration has been started with colleagues from Institute of Botany of the Academy of Science of the Czech Republic concerning the relationship between aboveground and belowground functional traits.

I focused on semi-natural grasslands because they represent priority habitat (6210* habitat according to 92/43/EEC Directive) since they host high biodiversity and represent an essential part of the cultural landscape resulted from centuries of land use (Dengler et al., 2014). Currently, the cessation of traditional managements practices (pastoralism and arable farming; Sebastià et al., 2008) is altering the ecosystem services (from fodder provision, soil stability to water and climate regulation; de Bello et al., 2010) that semi-natural grassland were used to provide. The effect of abandonment is promoting natural succession processes, with the expansion of shrub-lands and forests at landscape level (Malavasi et al., 2018), and the invasion of coarse tall grasses mostly with competitive stress-tolerant strategies such as species belonging to *Brachypodium* genus at community level (Catorci et al., 2011). Trait-based investigation that involves several approaches, i.e. studying aboveground and belowground functional traits variation along spatial and temporal gradient, using of intraspecific trait variability at population- and community-level, may provide a better understanding on how the ecosystem works, also in the light of vegetation prediction due to global changes.

In synthesis, in this thesis I focused on those topics that have been few investigated to date. I suggested an approach to disentangle assembly processes behind the same functional pattern (i.e. convergence or divergence); I tried to assess the relevance of intraspecific trait variation at population level for the most invasive species spreading in grassland community after land abandonment; I tried to investigate the relationship between aboveground and belowground functional traits at different scale (within habitat and across habitats), partitioning also the relative contribution of intraspecific trait variation and species composition to the community trait variation for belowground trait; finally, I assessed temporal shift of species diversity and their functional traits using permanent plots to understand how these changes could affect the relative ecosystem services and functioning.

In detail, the aims of this PhD thesis were:

Regarding aboveground traits along spatial gradient:

- 1) does relationship between the dispersion and the mean of a trait in a given community represent a useful approach to disentangling community assembly rules? (Chapter 1);
- 2) How does environmental condition variation affect intraspecific trait variability of the grass species *Brachypodium genuense*? (Chapter 2)

Regarding above- belowground traits along spatial gradient:

3) Do belowground functional traits show opposite pattern to aboveground across habitat (Chapter 3a) and within habitat? (Chapter 3b); moreover, what is the relative contributions of intraspecific trait variability and species composition on belowground functional trait variation? (Chapter 3b)

Regarding aboveground traits along temporal gradient:

4) How changed the community functional traits and species composition along temporal gradient? (Chapter 4)

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Is the relationship between trait dispersion and trait mean useful to reveal community assembly processes? A case study from sub-Mediterranean mountain grasslands

Alessandro Bricca¹, Luisa Conti², Maria Federico Tardella³, Andrea Catorci³, Marco Iocchi¹, Jean-Paul Theurillat⁴, Maurizio Cutini¹

Affiliation

¹Department of Science, University of Roma Tre, Rome, Italy

ale.bricca@gmail.com (<https://orcid.org/0000-0003-0202-6776>)

maurizio.cutini@uniroma3.it (<https://orcid.org/0000-0002-8597-8221>);

²Department of Botany, Faculty of Sciences, University of South Bohemia, Na Zlate Stoce 1, CZ-370 05 České Budějovice, Czech Republic.

luisa.conti@gmail.com (<https://orcid.org/0000-0001-8047-1467>)

³School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy

dtfederico.tardella@unicam.it (<https://orcid.org/0000-0002-4319-9131>);

andrea.catorci@unicam.it;

⁴Centre Alpien de Phytogéographie, Fondation J.-M. Aubert, Champex-Lac, Switzerland & Section of Biology, University of Geneva, Chambésy, Switzerland

Jean-paul.theurillat@unige.it (<https://orcid.org/0000-0002-1843-5809>)

Correspondence

Maurizio Cutini, Department of Science, University of Roma Tre, Rome, Italy

email: maurizio.cutini@uniroma3.it

Running head un unified approach of functional metrics

Abstract

Different processes may lead to the same functional pattern (convergence/divergence). Disentangling the processes may request assessing the relationship between community-weighted-mean (CWM) and trait-dispersion (FDis). We tested this hypothesis along elevation gradient in Mediterranean region. Contrary to the negative trait-elevation relationship in temperate mountains, due to cold temperatures at higher elevations, we hypothesized unimodal relationship in mediterranean mountain for drought' presence at lower elevations.

The location was Velino massif (Central Apennines). We collected species cover and we measured traits referred to LHS scheme along elevation gradient (1325-2375 m a.s.l.).

We performed generalized least square models between elevation and functional indices, for single and multi-traits, and linear model between indices. All indices showed unimodal relationship with the elevation, except for CWM_{SLA} .

At higher elevations lower CWM and FDis values suggested cold temperatures acting as abiotic filter for all traits. At intermediate elevations, on more unconstrained conditions, seed mass and plant height seemed shaped by biotic interaction (higher CWM and FDis). At lower elevation aridity seemed act as filters for seed mass, while plant height seemed shaped by abiotic and biotic filters, i.e. lower FDis values related to different CWM values. SLA variation may indicate that factors other than climate could affect this trait.

We suggested that trait-elevation relationship is climatic context, that each trait is affected by different biotic and abiotic processes. Finally, assessing relationship between functional indices for single traits, may be useful to identify assembly processes behind the same functional pattern for competitive effect traits, i.e. plant height and seed mass.

Keywords elevation gradient, functional traits, LHS scheme, CWM, FDis, biotic interactions, sub-Mediterranean grasslands

INTRODUCTION

Functional trait approach is increasingly recognized as useful tool for understanding mechanisms driving the species distribution (Lavorel & Garnier, 2002; Lavorel et al., 2011; Garnier et al., 2016; Vojtkó et al., 2017). Indeed, the functional approach has been effective in achieving a more mechanistic understanding of how species respond to environmental factors (Lavorel & Garnier, 2002; Mason et al., 2011; Tardella et al., 2017; Scolastrri et al., 2017), and has helped to reveal community assembly rules (Götzenberger et al., 2012).

According to niche theory, abiotic factors select only those species with a viable trait combination (“habitat filtering”; Keddy, 1992), promoting the coexistence of species with similar ecological preference (“functional convergence”; Weiher & Keddy, 1995; Cornwell & Ackerly, 2009). In contrast, negative biotic interactions are expected to constrain the overlap of ecological similarity between coexisting species (“limiting similarity”; MacArthur & Levins, 1967), and consequently the species will tend to diverge from each other (“functional divergence”). These processes might operate within a given plant community and the dominance of one process over the other will depend on the scale at which the communities are studied (de Bello et al., 2013b), on the given trait considered (Grime, 2006b) and on the intensity of the environmental conditions (Weiher & Keddy, 1995; May et al., 2013; Schellenberger Costa et al., 2017).

However, inferring processes from trait patterns has its unresolved issues. For example, researchers frequently fail to consider some filtering processes such as ‘weaker-competitor exclusion’. Indeed, negative biotic

interactions can also promote functional convergence, since highly competitive species may filter out weaker competitors (de Bello, 2012; Grime, 2006 b; Mayfield & Levine, 2010). Furthermore, environmental heterogeneity can lead to trait divergence, since functionally different species might occupy different environmental micro-habitats within the site (de Bello et al., 2012; Conti et al., 2017). Therefore, to shed light on the link between functional patterns and assembly processes, it may be useful to study not only the trait variation along the environmental gradient, but also the relationship between different functional metrics along the gradient.

The functional composition of a biological community is generally described by the mean and the dispersion of a given trait. We acknowledge that these metrics are mathematically correlated (Diaz et al. 2013), however only one study to date has attempted to quantify their relationship (Vojtko et al., 2017). We suggest that for traits directly related with competitiveness, analysis of their relationships could help reveal the underlying assembly process acting on a given community. For example, a high community mean value related to low functional dispersion value for plant height could be interpreted as a sign of weaker competitor exclusion, since taller species might exclude shorter species (Lepš et al., 2011; 2014). Contrary, a low community mean value related to a low functional dispersion value can indicate the dominance of environmental filtering, with the environmental constraint hindering the development of taller species. Therefore, by analysing the changes in the relationship between trait mean and trait dispersion at the community level along an environmental gradient, we are more likely to gain understanding of the process leading to the observed functional pattern. Elevation gradients are natural laboratories for studying these fundamental ecological issues (Körner 2007; Michalet et al., 2014; Rosbakh et al. 2015). In a temperate climate, low temperature seems to be the major limiting factor affecting plant performance at higher elevations (Körner, 2007; Michalet et al., 2014) leading to observed functional convergence (Dainese et al., 2012; de Bello et al., 2013a; Rosbakh et al., 2015). Instead, in Mediterranean mountains, temperature and precipitation gradients have opposite patterns and direction, that is, temperature decreases with elevation while precipitation increases (Theurillat et al. 2011). This implies that towards lower elevations, plant communities should be constrained more by summer drought, while plant communities at higher elevation should be more affected by winter frost (Pescador et al., 2014; Tardella et al., 2016).

In this paper, our aim was to investigate how community functional traits respond to an elevation gradient in a sub-Mediterranean climate context. To do so, we focused on plot scale to minimize the environmental heterogeneity and we evaluated the relationship of the community weighted mean (CWM) and the functional dispersion (FDis) with an elevation gradient, as well the relationship between CWM and FDis. We hypothesized that the variation in the climatic factors along the elevation gradient would affect both functional metrics. In particular, we expected that: i) functional convergence would be predominant at lower and higher elevations, under more strong environmental conditions; ii) functional divergence would be predominant at middle elevations under milder climatic conditions; iii) plant communities would show adaptations to increases in aridity and cold by shifting their mean trait values; and iv) assessing the relationship between CWM and FDis of a given functional trait would help us understand different assembly processes.

METHODS

Study Area

We focused on the vegetation of Monte Velino (2486 m a.s.l.), which is located in the Central Apennines (Abruzzo Region) in Italy. The massif is entirely composed of limestone (Petriccione, 1993). Climatically, the Velino massif belongs to the Mediterranean region, exhibiting a sub-Mediterranean bioclimate with a short summer drought period at low elevations and winter frost stress at higher elevations (Table 1). During the growing season (May-September), the mean temperature is around 17°C at 1,200 m a.s.l. and 8°C at 2,200 m, while the mean precipitation is around 350 mm and 460 mm, respectively (Fig. 1a-1b; Theurillat et al., 2011; Theurillat et al., unpubl.). The Velino massif is part of the Sirente-Velino Natural Regional Park (54391 ha) and is representative of the landscape dynamics occurring in the Mediterranean mountains in the last centuries (Malavasi et al., 2018).

Secondary dry grassland with *Bromus erectus*, *Carex humilis*, *Globularia meridionalis*, and *Sesleria juncifolia* dominates the lowest part of the area, up to approximately 2,000 m a.s.l. Above this elevation, the vegetation is mainly dominated by cryophilous swards with *Carex kitaibeliana* and *Sesleria juncifolia*, and includes alpine elements such as *Silene acaulis* and *Potentilla crantzii* (Petriccione, 1993).

| Termopluviometric station | 1 | 2 | 3 | 4 |
|---------------------------|--------|--------|--------|--------|
| Altitude (m a.s.l.) | 1450 | 1700 | 1950 | 2200 |
| T (°C) | 15.02 | 12.92 | 11.47 | 7.79 |
| T winter (°C) | 2.17 | 0.06 | -0.40 | -3.46 |
| MAT | 8.64 | 6.52 | 5.50 | 2.06 |
| P (mm) | 434.28 | 419.07 | 456.36 | 455.64 |
| PET (mm) | 89.53 | 85.40 | 78.86 | 72.17 |
| Aridity Index (P/PET) | 1.07 | 1.09 | 1.31 | 1.53 |

Table 1. Climatic characterization of the study area. Mean values of air temperature (T), sum annual precipitations (P), potential evapotranspiration (PET), aridity index during the growing season (May to September). T winter = mean values of air temperature during the winter season from November to February; MAT = mean annual temperature.

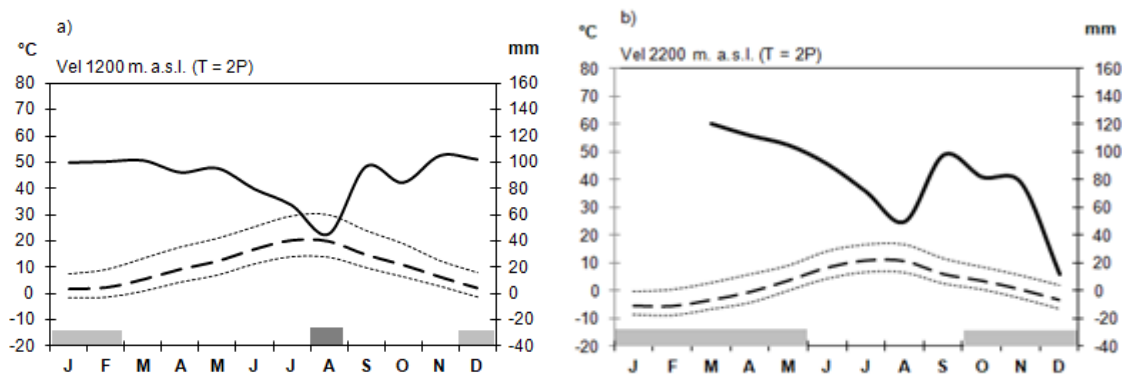


Fig.1. Lowest (a) and highest (b) termopluviometric station present in the study area (Velino Massif, Central Italy). Black dashed line represents mean monthly temperature. Grey dashed lines represent min and max monthly temperature. Black continuous line represent double the amount of precipitation for each month. Data are referred to 2006-2016.

Sampling of vegetation and functional traits

During the 2016, we revisited a selection of plots initially established in 2006, after that a monitoring project supported by Italian Ministry of Education, Universities and Research started (Fig. 2). To exclude inter-plot environmental heterogeneity along the elevation gradient, we selected only those plots on open calcareous grassland with an absence of domestic grazing, located in areas with a south-west aspect (227 ± 43 degrees), and with the same slope values (33 ± 4 degrees). This resulted in 45 plots (2x2 m) with fairly homogenous environmental conditions intra-plot, positioned from 1,325 m a.s.l. to 2,375 m a.s.l. From May to August for each plot, species presence was recorded and their relative cover was visually estimated using the Braun-Blanquet method. Prior to analysis, these records were transformed to percentage values as follows: +: 0.1%, 1a: 2.5%, 1b: 5% 2a: 10%, 2b: 20%, 3a: 31.25%, 3b: 43.75% 4a: 56.25%, 4b: 68.75%, 5a: 81.25%; 5b: 93.75%. We quantified species traits occurring at different elevations, focusing on the most abundant species, given that the trait value of dominants is expected to largely affect community assembly and functioning (“mass ratio” hypothesis; Grime 1998). To do so, we divided the elevation gradient into four sections of almost 250 m. The first section (1325-1575 m asl) had 9 plots, the second (1575-1825 m a.s.l.) had 10, the third (1825-2075 m asl) had 11 and the last section (2075-2375 m a.s.l.) had 15. We followed the guidelines proposed by Pakeman & Quested (2007), but rather than using the species cover values for each plot, we pooled together the values of all the plots in each elevation belt. We selected all those species whose relative cumulative cover reached 80% of the total vegetation cover in each elevation belt (Appendix 1). This resulted in a total of 50 species, partitioned into 11 for the first section, 10 for the second, 12 for the third and 17 for the last one (Appendix 2).

We measured the traits indicated in Westoby’s widely used leaf-height-seed (LHS) strategy scheme (Westoby, 1998), namely, vegetative plant height (H), specific leaf area (SLA), and seed mass (SM). For each of the 50 species, we noted plant height (cm) in 15 individuals and SLA (mm^2/mg) in 10 healthy, fully expanded leaves

from 10 individuals. For seed mass (mg), we collected at least 2 seeds per plant, from no fewer than 3 individuals. All the individuals were gathered in the mid-point of each elevation section, where the topographic factors of the plots were the same. They were measured according to an internationally recognized standardized trait measuring protocol (Perez-Hargundegay et al., 2013).

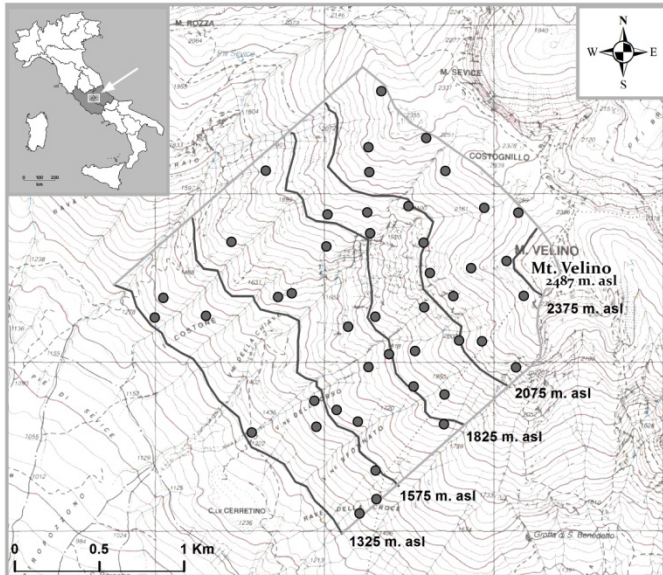


Fig.2. The rectangle represents the study area (Velino Massif, Central Italy) and continuous line represent the 100 m elevation bands of the MIUR Project. Dots are the subset of 45 permanent plots of the MIUR Project selected for the sampling.

Functional indices

We computed the community weighted mean (CWM; Garnier et al., 2004) and functional dispersion (FDis; Laliberté and Legendre, 2010) indices for each plot using species cover at plot level and trait values collected at the mid-point of each elevation section. CWM correspond to the average trait value in a community weighted by the relative abundance of the species carrying each value (Garnier et al. 2004). It is widely used to measure the relationship between traits and the environmental conditions, and thus serves to quantify how the mean trait values in a community shift due to environmental selection. FDis quantifies the degree of functional dissimilarity within the community (Laliberté and Legendre, 2010). It is calculated as the weighted mean distance, in multidimensional trait space, of individual species from the weighted centroid of all species, where weights correspond to species relative abundances (Laliberté and Legendre, 2010). Compared to other functional dispersion indices, the FDis index is not strongly influenced by outliers, nor is it affected by species richness. High/low values are expected to indicate a high/low degree of niche differentiation, i.e. functional divergence/convergence (Laliberté and Legendre, 2010). We computed the community-weighted mean for single traits (hereafter CWM_H , CWM_{SLA} , CWM_{SM}), and functional dispersion for single (hereafter $FDis_H$, $FDis_{SLA}$, and $FDis_{SM}$, respectively) and multi-traits ($FDis_{LHS}$).

Gower distance, which also provides the trait standardization necessary when computing the multi-trait index, was used to calculate FDis indices. All functional indices were computed with the dbFD function in the R package, “FD” version 1.0 (Laliberté et al., 2014).

Data analysis

Following Bässler et al. (2016), we used elevation (m a.s.l.) as a proxy for temperature and precipitation along the elevation gradient. To test the effects of the environmental gradient on species richness and functional indices, we set up a linear model for each index using elevation as an explanatory variable. We introduced a quadratic term for the explanatory variable, as we hypothesized that the response of communities to elevation in a sub-Mediterranean climate is not necessarily linear. After visual inspection, we noted that residuals showed heteroscedasticity, and therefore chose to fit the generalized least square models (GLSs) using the *nlme* package version 3.1 (“gls” function), choosing the most appropriate variance structure by the minimum AIC criteria (Zuur et al., 2009).

Finally, to identify the process behind the functional pattern, we performed a simple linear model to analyse the relationship between CWMs and single FDis indices. We used FDis indices as explanatory variables and CWMs as response variables. These model assumptions were checked by visual inspection. Species richness and CWM indices were log-transformed before the analysis to improve the normality of residuals.

RESULTS

We observed a negative linear relationship between species richness and elevation (slope value: -0.001; p-value < 0.01), with species number slowly decreasing towards higher elevations (Fig. 3a). We found a unimodal relationship between FDis_{LHS} (slope value: -1.24; p-value < 0.01) and elevation, with higher values at middle elevations, and lower value at both end of the gradient (Fig. 3b).

Both CWM_H and FDis_H showed a unimodal pattern in relation to elevation, with the highest values at the middle elevations (CWM_H; slope value: -1.01; p-value < 0.01 and FDis_H; slope value: -0.13; p-value < 0.05; Fig. 4a-b). We found a unimodal relationship (slope value: -0.18; p-value < 0.001; Fig. 4c) between the two functional metrics quantified.

In the case of SLA, we discovered a unimodal relationship with elevation for FDis (slope value: -0.14; p-value < 0.05; Fig. 4d), whilst the CWM showed a linear decreasing pattern (slope value: -0.001; p-value < 0.05; Fig. 4e). We did not find any significant relationship between indices.

For seed mass, we observed the same unimodal pattern along the elevation gradient for both the CWM_{SM} index (slope value: -1.08; p-value < 0.01; Fig. 4g) and the FDis_{SM} index (slope value: -0.86; p-value = 0.001) (Fig. 4h). The relationship between the indices was positively linear (slope value: 0.05; p-value < 0.001; Fig. 4i). Further information on the models is reported in Appendix 3.

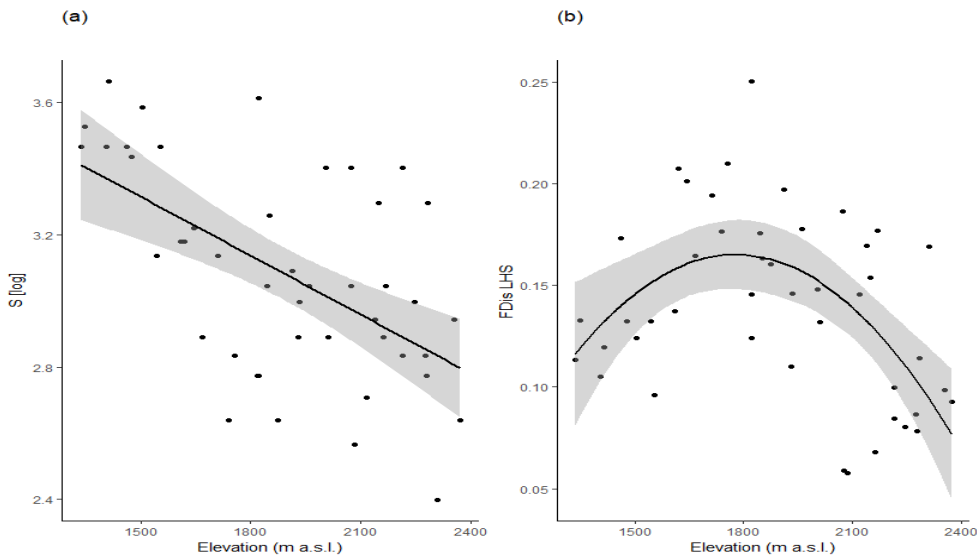


Fig. 3. Generalized least square models showing linear relationship for species richness and elevation (a) and quadratic relationship for multi-trait functional dispersion and elevation (b).

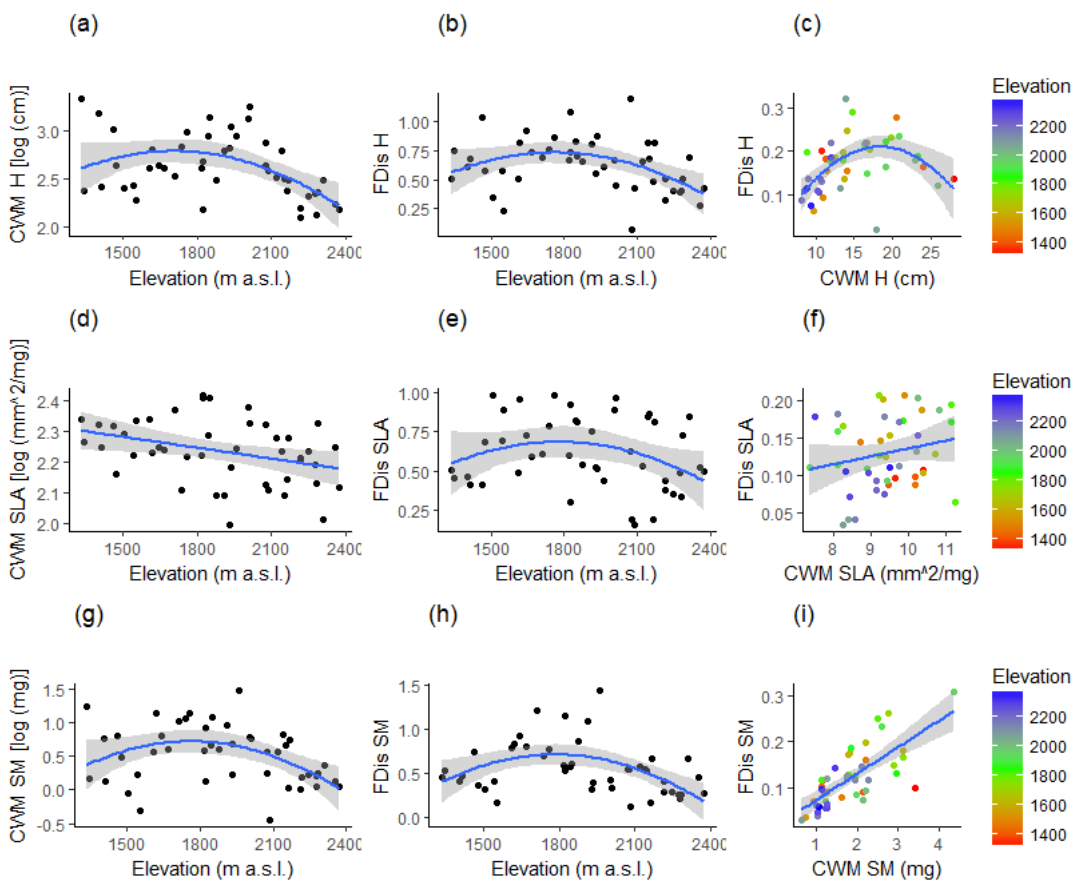


Fig. 4. Generalized least square models for LHS scheme and linear model between functional metrics. CWM (a) and FDis index (b) showed a quadratic relationship between elevation and plant height. Community mean values of SLA showed a linear relationship with the elevation (d). A quadratic relationship resulted with the FDis index (e). For both functional indices of seed mass, the elevation showed a quadratic relationship (CWM; g) (FDis index; h). Linear model between functional indices showed a quadratic relationship for plant height (c) and linear relationship for SLA (f) and seed mass (i).

DISCUSSION

Multi-trait and richness patterns along the gradient

The multi-trait approach allowed us to detect only a general functional pattern, that is, convergence towards lower and higher elevation. Interestingly, the high values of species richness at the lower part of the elevation gradient in combination with low $FDis_{LHS}$ values suggested the presence of a high number of coexistence species sharing similar functional traits (i.e. lower niche differentiation and high niche overlap; Mason et al., 2005). This pattern might suggest that there is aboveground trait redundancy in the community (de Bello et al., 2009), and that the differentiation in their belowground niche may allow the coexistence. Indeed, species co-existence in a given assemblage could not be maintained if their own niches are completely overlapped (Gause 1964; MacArthur & Levins, 1967).

Above 2,000 m a.s.l., we found a decrease of species richness accompanied by a decrease of $FDis_{LHS}$: these grasslands are characterized by species-poor communities with a narrow number of functional strategies. Therefore, at these altitudes, severe environmental conditions act as an abiotic filter on the functional strategies as well as on species composition, selecting only those species adapted to cold temperature (Theurillat et al., 2011).

Patterns in plant height (H)

Communities at higher elevations are constrained into a lower variety of plant height values (Fig. 4b), and at the same time these communities present a relatively low value of CWM (Fig. 4a). Conversely, lower to middle elevation communities are characterized by higher values of $FDis$ while having a certain degree of variability in CWM values. This is particularly evident in Figure 4c, as all the higher elevation communities are in the bottom left corner of the plot, while all the others are distributed in the rest of the plots (please refer to the colour version of this figure). This seems to suggest the presence of multiple processes along the elevation gradient: habitat filtering (low CWM, low $FDis$) may be especially important in communities at the highest elevations, limiting similarity at middle elevations (high CWM, high $FDis$), while at low elevation weaker-competitor exclusion (high CWM, low $FDis$) and habitat filtering (low CWM, low $FDis$). These findings are mostly in line with previous suggestion stating that abiotic factors will be more dominant in the structuring of communities in areas under more severe environmental conditions (Weiher & Keddy, 1995), while negative biotic interactions will be the dominant processes shaping communities in less constrained environmental conditions (Mason et al., 2011; Lhotsky et al., 2016).

In grassland communities at higher elevations, dominated by low temperature, small size is certainly the most prominent adaptation of plants (Theurillat et al., 2011; Dainese et al., 2012; de Bello et al., 2013a): by reducing their stature, plants are able to benefit from the soil heat accumulated during the day (Körner, 2003). Furthermore, short plants tend to be more protected from desiccation by snow cover (Grime, 2006 a). The co-presence at lower elevation of biotic filtering and processes that limit similarity may be due to the water retention characteristics of the soil, which partly determine the level of aridity experienced by plant

communities. Indeed, unlike temperature, aridity can be highly variable because of its dependence on soil conditions, as the water available for plants also depends on the soil's ability to retain water (Cassel & Nielsen, 1986). Therefore, where the soil is more prone to retain water, it could allow the growth of a higher plant canopy (Shao et al., 2008), which in turn can filter out the shorter species. For the plots where we observed divergence in plant height, especially in the case of lower elevation plots, aridity might not be strong enough to constitute an environmental filter, and thus limiting similarity would dominate. This tendency at lower elevation is in line with previous studies performed in Mediterranean climatic condition (Gross et al., 2013; Tardella et al., 2016; Nunes et al., 2017), since drought tend to select a lower range of plant height towards small size.

Patterns in specific leaf area (SLA)

The absence of a clear trend along the elevation gradient prevents us from clearly distinguishing assembly processes (Fig. 4e, d). This is particularly evident in Fig. 4f, where the relationship between the two functional metrics does not show any clear pattern (please refer to the colour version of this figure). For communities at high elevations, we saw a tendency to convergence towards relatively low CWM values, which might indicate a prevalence of environmental filtering. In this sense, our findings are in line with previous works focused on the alpine environment (de Bello et al., 2013a; Rosbakh et al., 2015). Lower SLA values might prove to be an advantage when facing lower temperatures, as lower values are related to higher content of protein and secondary compounds which increase the leaf resistance to freezing (Poorter et al., 2009).

The pattern of SLA in association to climatic factors, such as temperature and precipitation (see Borgy et al. 2017, and references within) or aridity (Nunes et al., 2017), is still unclear. Moreover, previous studies have found contrasting results: an absence (Dainese et al., 2012) or a negative (de Bello et al., 2013a) relationship. The higher variability in the CWM_{SLA} along our elevation gradient, could be explained by the fact that local factors (such as nutrient availability) affect SLA more than climate (Hodgson et al., 2011; Vojtkó et al., 2017). The lack of a link between SLA and any competitive assembly mechanisms along the gradient could be due to the relationship of SLA with species competitiveness. Although SLA is sometimes considered to be positively correlated with relative growth rate and competitive ability (Westoby, 1998), previous studies have linked this trait to competitive responses, that is, the ability to avoid being suppressed by other individuals, rather than competitive effects, that is, the ability to suppress other individuals (Goldberg & Landa, 1991; Kraft et al., 2014; Conti et al., 2018). Species competitive responses will be context dependent and therefore whether the SLA of communities is shaped by competitive processes will depend on how the single species in the community respond to the specific environmental conditions and to the specific biotic interaction, making it difficult to find a clear pattern along elevation gradient.

Patterns in seed mass (SM)

Our observation of lower CWM_{SM} at lower elevation seems to contrast with the suggestion that under arid environments species should exhibit larger seeds than species from more mesic environments (Baker, 1972).

Larger seeds provide increased nutrition and are expected to develop into larger seedlings with increased changes of survival, particularly in arid environments (Moles & Westoby, 2006). However, other studies found an absence of such a pattern (Tardella et al., 2016; Nunes et al., 2017) or even the opposite, that is, decreases in seed mass with increases in aridity (Harel et al., 2011). These contrasting findings indicate that a clear relationship between seed mass and aridity has yet to be confirmed. Contrary, the decline of seed mass with increasing elevation is a common pattern (Grime, 2006 a; Dainese et al., 2012; de Bello, 2013a). It has been explained as an environmental response to lower temperatures, which may reduce seed growth (Körner, 2003), and to the short growing season that may reduce the time for seed development, therefore reducing the mass of mature seeds (Baker, 1972).

Our results indicate that in both low and high elevation communities, lower values of FDis are related to low values of CWM (Fig. 3i), suggesting a filter due to aridity and cold at lower and higher elevation, respectively. Seed mass is also related to seedling competition (Westoby, 1998; Grime, 2006 b). Therefore, in the middle elevations, higher FDis and higher CWM might indicate the prevalence of limiting similarity.

CONCLUSIONS

To the best of our knowledge the elevation gradient along sub-Mediterranean mountains has been for the most part neglected, contrary to alpine and temperate mountains. We found that the relationship between elevation and functional patterns is strongly dependent on the climatic context, and in a sub-Mediterranean context this relationship fits a unimodal trend.

We suggest that assessing the relationship between the “mean” and “dispersion” of functional trait in a community could help to identify the assembly process behind the same functional pattern, at least for traits related to the competitive effects. However, further investigations are needed to better understand how are related these indices in the biological communities.

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Context-dependent variation of SLA and plant height of a dominant, invasive tall grass (*Brachypodium genuense*) in sub-Mediterranean grasslands



Federico M. Tardella^a, Alessandro Bricca^b, Karina Piermarteri^{c,*}, Nicola Postiglione^c, Andrea Catorci^a

^a School of Biosciences and Veterinary Medicine, University of Camerino, via Pontoni 5, I-62032 Camerino (MC), Italy

^b Department of Sciences, University of RomaTre, viale Marconi 446, I-00146 Rome (RM), Italy

^c School of Advanced Studies, University of Camerino, via Lili 55, I-62032 Camerino (MC), Italy

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ABSTRACT

The cessation of semi-extensive farming and the abandonment of settlements are threatening European pastoral landscapes and their biodiversity, affecting species assemblage and ecosystem functioning. This process typically involves invasion by coarse tall grasses, mostly with competitive stress-tolerant strategies, and rapid development of mono-dominance stands. A key species in this respect is *Brachypodium genuense* (DC.) Roem. et Schult., an endemic, rhizomatous, tall-grass that grows in the semi-natural grasslands of the Italian peninsula over 1200–1400 m a.s.l.

We hypothesized that *B. genuense* was able to spread and dominate the grassland communities subjected to a wide range of environmental conditions through its broad spectrum of performances. In fact, it has considerable morphological variability, mirrored by high variations of traits, namely Specific Leaf Area (SLA), individual height, and tussock cover values. The study area encompassed the Monti Sibillini National Park (central Italy). We found that in more productive conditions (deeper soil, medium/high pH values, north-facing slopes, and “conservative” landforms) populations of *B. genuense* had higher SLA, plant height and cover values than in low productive conditions (south-facing slopes, shallow soils, lower pH values). This indicates a fast-growing strategy based on a high rate of resource acquisition and use, as well as high competitive ability for aboveground resources such as light under productive conditions. Under unproductive and/or dry conditions decreased SLA, plant height and cover value indicate a slow-growing strategy with high conservation and low rate of resource acquisition. In addition, we found that a very high amount of nitrogen in the soil might have a detrimental effect on *B. genuense* individuals, decreasing the SLA values, thus requiring the plant to change its growth strategy. Finally, we inferred that the observed variability in SLA might have a paramount role in the invasive/dominant behavior of *Brachypodium genuense*.

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1. Introduction

For long time, anthropogenic actions such as deforestation, burning, grazing and mowing created and maintained pastoral systems, which are now considered a hot spot for European biodiversity (Pärtel et al., 2005) and provide several ecosystem services and socio-economic benefits (Reynolds et al., 2005). However, the cessation of semi-extensive farming and the abandonment of mountain settlements are threatening European pastoral landscapes and their biodiversity (Sebastià et al., 2008). In particular,

farming cessation or livestock pressure below the theoretical carrying capacity of pastures affect species assemblage and ecosystem functioning, leading to vegetation changes (Peco et al., 2006). At the community level, this process typically fosters invasion by coarse tall grasses mostly with competitive stress-tolerant strategies, and rapid development of mono-dominance stands (Louault et al., 2002). In fact, these plants tend to dominate the community thanks to particular sets of traits such as tall canopy, extensive lateral spread, clonal integration strategy (allowing transport of resources from original ramets growing in non-stressful microhabitats to subsequent ramets in stressful conditions through a connecting rhizome or stolon; Alpert, 1996) and high litter deposition (Grime, 2001). Moreover, the spread of tall grasses leads to changes in resource availability and alterations in spa-

* Corresponding author.

E-mail address: karina.piermarteri@unicam.it (K. Piermarteri).

tial and temporal niche occupation (Muller, 2002). This in turn affects seed germination and the establishment and growth of other species, and results in a loss of species richness (Muller, 2002; Catorci et al., 2011a; Peco et al., 2012; Szentes et al., 2012). In this regard, *Deschampsia* (Jendrišáková et al., 2011), *Calamagrostis* (Házi et al., 2011), *Sesleria* (Kuzmanović et al., 2009) and *Brachypodium* (Bonanomi et al., 2013) emerged as the most widespread dominant tall grass genera in Europe. A detrimental species in this respect is *Brachypodium genuense* (DC.) Roem. et Schult, an endemic, rhizomatous, competitive-stress tolerant tall grass (sensu Grime, 2001). *Brachypodium genuense* occurs in the semi-natural grasslands of the Italian peninsula, over 1200–1400 m a.s.l. (Camiz et al., 1991). Its spread is primarily fostered by the cessation of traditional management practices such as grazing and mowing (Catorci et al., 2011a,b). In fact, because of its silica-rich and hairy leaves, domestic herbivores feed on it only in conditions of overstocking (Catorci et al., 2014a). Moreover, clumps of *Brachypodium* enclosed in a multi-species community are visited by herbivores, and consequently have a higher chance to be defoliated (neighbor association). On the opposite, large stands of *Brachypodium* are avoided (Canals et al., 2017). Catorci et al. (2011b, 2012) proved that the spread of *B. genuense* reduces light irradiance at the ground level, as well as soil pH, temperature and water content. Moreover, in accordance with the phenological “mid-domain hypothesis” (Morales et al., 2005), its dominance alters the temporal niche partitioning within the plant community, enhancing the “edge” effect in the flowering patterns of the subordinate and accidental species (Catorci et al., 2014b). In addition, the spread of *B. genuense* dramatically reduces the nutrient value of pastures (Vitasović Kosić et al., 2014), thus negatively affecting shepherding (Catorci et al., 2014a). In fact, it has been shown that low forage quality negatively affects rumen morpho-physiological performance (Scocco et al., 2012, 2013, 2016). Similarly, it has also been proven that *Brachypodium* encroachment reduces habitat suitability for wild herbivores (Corazza et al., 2016). Consequently, management of *B. genuense* populations is a major goal for both biodiversity and socio-economic reasons. There is a need for greater understanding of the ecological factors that allow its spread and of the relationship between *B. genuense* functional features and environmental constraints. The latter is a key issue, since traits reflect the trade-offs among different functions within a plant (Violle et al., 2007). Moreover, variations in resource availability have been shown to determine inter- and intra-specific variations of traits (Albert et al., 2010; Auger and Shipley, 2013). Therefore, the assessment of intra-specific trait variation may be a paramount tool in predicting species performances and in understanding species ecological behavior in the light of environmental heterogeneity (Wellstein et al., 2013).

In this view, specific leaf area (SLA, one-sided leaf area per unit of dry mass) and plant height (the shortest distance between the upper boundary of the main photosynthetic tissue on a plant and the ground level) are two of the major traits that reflect species ecological strategies (Westoby, 1998). SLA is a very useful leaf trait for estimating the responses of individuals, species, communities or whole ecosystems to environmental variations and resource gradients (Díaz et al., 2007; Pérez-Harguindeguy et al., 2013). In fact, it is related to the rate of resource investment, and thus by studying it we can understand more clearly the trade-off between resource acquisition and resource conservation strategies. Moreover, it is an indicator of species position along resources gradients (Pérez-Harguindeguy et al., 2013), since variation in local resources has been proven to drive SLA intra-specific changes (Messier et al., 2010). Generally, species and/or individuals with high SLA are found in more favorable conditions, while harsher conditions foster individuals with lower SLA and plant height (stress-tolerant perfor-

mances) (Kichenin et al., 2013). In fact, leaves with high SLA (lower leaf thickness and/or density) produce less photosynthetic machinery per unit area, and therefore utilize diffuse radiation (low light intensity) more efficiently (Sefton et al., 2002); leaves with high SLA also have greater relative growth rates (Shipley, 2002). Conversely, leaves with low SLA (greater leaf thickness and/or density) utilize high irradiance more efficiently, and are more tolerant to water and nutrient shortage due to their thicker cuticle layers and their slower leaf turnover rates (Fonseca et al., 2000).

Plant height is another easily measurable trait and a key plant strategy related to reproduction (Pérez-Harguindeguy et al., 2013). In fact, it indicates the ability of plants to compete for light and the resource investment of individuals for stem biomass construction; measured at the individual level, this trait reflects plant fitness and indicates the plant responses to environmental constraints, since it is associated with the ability to tolerate or avoid environmental stresses (Violle et al., 2007).

Since very little information is available about the interplay between stress intensity and SLA/plant height of dominant tall grasses, especially in a Mediterranean type climate, we carried out a study focused on responses of morphological traits (SLA and plant height) and cover value of *B. genuense* to fine-scale environmental variations in central Apennines (Italy). In particular, we sought to understand the rate and direction of the multi-factor, context-dependent, intra-specific trait variation and their relationship with different environmental constraints. To address these issues, we set the following questions: i) do topographic and soil features affect SLA and plant height of *B. genuense* and if so, in what direction? ii) which environmental variables, singly or in combination, have stronger influence on intra-specific trait variation?

We also wondered if intra-specific SLA variation across productivity gradients highlights a shift of the species strategies from a slow-growing strategy in unproductive conditions to a fast-growing strategy in productive environments, highlighting variability in leaf morphology as a possible complementary mechanism in the dominant behavior of the species.

2. Material and methods

2.1. Study area

The study area encompasses the Monti Sibillini National Park (central Italy; central coordinates 42° 49' 26" N, 13° 16' 32" E), and ranges from 1400 m to 2250 m a.s.l. This area, of about 20,000 ha, is mainly characterized by limestone bedrock. The plant landscape consists of forest ecosystems belonging to the *Fagetalia sylvaticae* Pawlowski in Pawlowski, Sokolowski & Wallisch 1928 order, and grassland communities belonging to the *Brometalia erecti* Br.-Bl. 1936, *Seslerietalia tenuifoliae* Horvat 1930, *Nardetalia strictae* Oberdorfer ex Preising 1949 and *Caricetalia davallianae* Br.-Bl. 1949 orders. The upper timberline runs at 1800–1900 m a.s.l. (Catorci et al., 2011a). The soils of the pastoral ecosystems are quite shallow (5–50 cm), with acid/sub-acid to neutro-alkaline pH, a medium to high amount of organic matter and sandy-loamy texture with high debris content (Giovagnotti et al., 2003). Regarding climatic features, the study area lies within the Temperate region, near the border of the Mediterranean one. Consequently, an alternation of winter cold stress and summer drought stress, with different intensities, depending on the elevation gradient and landform factors, characterizes the system (Rivas-Martínez and Rivas-Saenz, 1996–2016). Annual average temperature ranges from 3.5 to 7.0 °C, with winter average temperature of 1.0–4.5 °C and summer average temperature of 7.0–10.5 °C. Average annual precipitation ranges from 1300 to 1600 mm, with two seasonal peaks in late spring and autumn and rainfall decrease in sum-

mer (240–280 mm). A very high grazing pressure characterized the pastoral landscape for centuries, but the last decades have seen a marked trend of grazing cessation and mountain farming abandonment, and thus several sectors of the National Park are currently abandoned and undergoing vegetation recovery and dynamic processes.

2.2. Data collection

We conducted a preliminary survey of the *B. genuense* populations using bibliographic data, Google's Satellite pictures and transects which followed roads and pathways. We outlined the results of this survey on a topographic map (scale of 1:10,000), then overlaid it with a grid with cells of 500 m × 500 m. Using a stratified sampling design, we divided the altitudinal range of *B. genuense* into four 200-m-wide elevation classes (from 1400 to 2200 m a.s.l.) and, in each of them, we randomly selected a number of cells of 500 m × 500 m including one or more patches of *B. genuense*, proportional to the total number of cells where *B. genuense*-dominated patches occurred in that elevation class.

In each selected cell, we chose a patch covered by *B. genuense* and then we laid on that patch one linear transect (with variable length depending on patch dimension) parallel to the slope, to account for possible gradients due to the largely unpredictable great variety of fine-scale environmental conditions, for example micro-topography, soil depth, soil pH and nitrogen content in the soil. Along each transect we laid at least two 20 cm × 20 cm plots (221 in all). Consecutive plots were 100 cm apart from each other; the first one was positioned 50 cm within the patch limits in order to avoid including young individuals. The number of plots per transect depended on transect length. We chose a fine scale since, as stated by Lajoie and Vellend (2015), environmental variation on a small spatial scale (due to soil properties) represents the main driver of intra-specific variation in SLA and plant height. In each plot we collected data on elevation (m a.s.l.), aspect (azimuth degrees), slope angle (vertical degrees), landform (used as a categorical variable with seven levels: slope; concave water drainage surface, hereafter named “impluvium”; hollow surface on slope; hollow on semi-flat surface; convex surface straddling a watershed, hereafter named “watershed”; semi-flat surface; flat or semi-flat part of a step in a terraced slope, hereafter named “tread”), soil pH (measured in three points per plot on the same day using the PCE Instruments PH20S field pH-meter; measurements taken in each plot were then averaged) and soil depth (measured using a graduated pole), *B. genuense* cover percentage (visually estimated) and height (distance between the upper boundary of the main photosynthetic tissues – excluding inflorescences – on a plant and the ground level, measured in five locations inside each plot; measurements taken in each plot were then averaged). In addition, we collected one soil sample in each plot (up to 30 cm depth) to determine total soil nitrogen content (g kg^{-1}). Soil samples were analyzed by the Marche Region agrochemical analysis and research laboratories using the Dumas' (1831) method, according to the methodological standards established by Italian Ministerial Decree 13 Sep. 1999, modified by Italian Ministerial Decree 25 Mar. 2002. In each plot, 10 leaves of *B. genuense* were randomly collected (2210 samples in total), disregarding leaf age or the possible occurrence of leaves with symptoms of pathogens or herbivore attacks, to avoid any source of subjectivity in sampling. In order to preserve the leaves, we followed the standardized protocols described by Pérez-Harguindeguy et al. (2013). Leaves were wrapped in moist paper and kept in a cooler bag; then, we placed the cooler bag in the shade to minimize transpirational water loss. Afterwards, within each set of samples (10 leaves), one leaf was chosen randomly (221 leaves in all) among those that complied with the characteristics requested by the protocols (Pérez-Harguindeguy et al.,

2013), namely non-senescent fully expanded and hardened leaves. The leaf area (mm^2), hereafter LA, was obtained with the use of a desktop scanner and the “Leaf Area Measurement” software, University of Sheffield (UK) version 1.3, and leaves were dried for 48 h at 90 °C for determination of leaf dry mass (mg), hereafter LDM. SLA was calculated for individual leaves, as the ratio of LA to LDM ($\text{mm}^2 \text{mg}^{-1}$). Data were collected between 15th July and 5th August 2015, when *B. genuense* was blooming and fully developed.

2.3. Statistical analysis

As a preliminary step, following Warren II (2008), we converted aspect azimuth from the 0–360 compass scale to a 0–180 linear scale, giving northerly aspect (the shadiest one) a value approaching 0 and southerly aspect (the sunniest one) a value approaching 180. This transformation also converted east and west azimuth degrees so that they were equally distant from north. Moreover, as south–south-west facing slopes are the warmest aspect, we shifted the aspect azimuth to a minimum on north–north-east slopes (22.5°) and a maximum on south–south-west slopes (202.5°).

To highlight possible correlations between response variables (SLA, plant height and cover), we calculated Spearman's rank correlation coefficients (Spearman's rho), and tested significance of correlations, applying Bonferroni correction for multiple tests (significance threshold = 0.017).

To assess the influence of the environmental variables on *B. genuense* features, we standardized the *B. genuense*-related variables (SLA, plant height, and cover) and performed redundancy analysis (RDA) (Borcard et al., 2011) on the “relevés-by-standardized *B. genuense*-related variables” matrix, constrained by the environmental variables (elevation, aspect, slope angle, landform, soil depth, pH, and nitrogen content). Landform was treated as a categorical variable, while elevation, aspect and slope angle, soil depth, pH, and total nitrogen content were treated as quantitative variables.

We tested RDA models and RDA axes using permutation tests (999 iterations). To detect possible strong correlations among explanatory variables in the RDA models, we explored linear dependencies by computing the Variance Inflation Factors (VIFs), taking into consideration that VIFs above 10 justify a reduction of the number of explanatory variables (Borcard et al., 2011). In case of VIFs higher than 10 we used the forward selection procedure, to exclude variables that did not contribute significantly ($P \geq 0.05$) to the explained variation (Borcard et al., 2011).

To assess the contribution of explanatory variables (singly or in combination) to the total variability of the response variables data set, the total variance of each response variable was partitioned into fractions by partial RDAs. We calculated adjusted R^2 values to produce unbiased estimates of the contributions of the independent variables to the explanation of the response variables (Peres-Neto et al., 2006). To test the significance of the adjusted R^2 (namely, whether each independent fraction exhibits a significant influence on cover data), we applied a permutation test with 1000 permutations, in accordance with Legendre and Legendre (1998).

To perform all statistical analyses, we used R software, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>). To perform Spearman's correlation and tests for significance of correlation we used stats R-package, version 3.0.2 (cor.test function), while for variable standardization, RDAs, tests of RDA models and RDA axes, VIF computation, forward selection of variables and variance partitioning we used vegan R-package, version 2.0-9 (rda, anova.cca, vif.cca, ordistep, and varpart functions).

Table 1

Descriptive statistics of specific leaf area, mean height of individuals, and cover percentage of *Brachypodium genuense* and quantitative topographic and edaphic variables, measured in 20 cm × 20 cm plots ($n = 221$).

| Response variable | Min. | Max. | Mean (SD) | Median (IQR) |
|-----------------------------------------------------|-------|-------|-------------|--------------|
| Specific leaf area ($\text{mm}^2 \text{mg}^{-1}$) | 8.9 | 36.4 | 17.8 (3.3) | 17.4 (3.9) |
| Plant height (cm) | 3.0 | 65.0 | 26.2 (10.3) | 25.0 (13.0) |
| Cover (%) | 2.0 | 100.0 | 64.9 (29.1) | 70.0 (45.0) |
| Explanatory variable | Min. | Max. | Mean (SD) | Median (IQR) |
| Elevation (m a.s.l.) | 1,468 | 2,154 | 1803 (217) | 1799 (487) |
| Aspect (azimuth degree) | 12.5 | 180 | 99.8 (47.8) | 95.5 (82.0) |
| Slope angle (vertical degree) | 1 | 70 | 21 (11) | 20 (15) |
| Soil depth (cm) | 3.0 | 100.0 | 38.4 (21.0) | 35.0 (29.0) |
| pH | 3.3 | 7.2 | 5.5 (0.9) | 5.7 (1.7) |
| Soil nitrogen content (g kg^{-1}) | 5.4 | 14.8 | 8.4 (2.1) | 7.8 (2.9) |

Max. – maximum; Min. – minimum; IQR – interquartile range; SD – standard deviation.

3. Results

SLA of *Brachypodium genuense* ranged from 8.9 to $36.4 \text{ mm}^2 \text{ mg}^{-1}$; plant height ranged from 3 to 65 cm; cover values ranged from 2 to 100%. Table 1 shows descriptive statistics of trait values and quantitative environmental variables. The relative frequencies of landforms were: slope, 43.4%; “watershed”, 14.0%; “tread”, 13.1%; hollow surface on slope, 10.0%; semi-flat land, 9.1%; “impluvium”, 6.3%; hollow surface on semi-flat land, 4.1%. The Spearman rank correlation analysis showed positive significant relation between SLA and plant height (Spearman’s $\rho(219) = 0.40$, $P < 0.001$), cover percentage and plant height ($\rho(219) = 0.64$, $P < 0.001$) and SLA and cover percentage ($\rho(219) = 0.27$, $P < 0.001$).

The total explained variance of the response variables, constrained by the environmental variables, was 34.1% ($P = 0.001$). The VIFs of all variables were below 10, thus we included all of them in the following analyses.

Variation partitioning showed that topographic variables (elevation, aspect, slope angle, and landform) and edaphic variables (soil depth, pH, and nitrogen content) explained 13.39 and 10.43% of the variability, respectively ($P = 0.001$). The common variance explained by the two sets of variables was 10.33%. Among the topographic variables, landform (adj. $R^2 = 0.054$; $P = 0.001$) and the joint effect of landform and elevation (adj. $R^2 = 0.069$) gave the greatest contributions, while soil depth (adj. $R^2 = 0.111$; $P = 0.001$), pH (adj. $R^2 = 0.062$; $P = 0.001$), and the joint effect of nitrogen and soil depth (adj. $R^2 = 0.063$) better explained the variance in response variables (Table 2).

The first axis of the RDA performed with all environmental variables as explanatory variables explained 88.6% ($P = 0.001$) of constrained variance and was linked mainly to elevation and soil depth (positive values); the second axis explained 9.6% of the variance ($P = 0.001$) and was related to southerly aspect (positive value) and soil pH (negative value). The third axis was mainly linked to “slope” landform and southerly aspect (negative values) (Table 3) with 1.8% of variance explained ($P = 0.092$). SLA, plant height and cover values of *B. genuense* had a positive relation with elevation, soil depth, slope angle, pH and “watershed”, “impluvium”, and “hollow surface on slope” landforms, while they had a negative relationship with soil nitrogen content and “semi-flat” and “tread” landforms (Fig. 1).

4. Discussion

4.1. Overall pattern

The observed common response pattern of traits to environmental features suggests that environmental constraints affected SLA and plant height/cover values in the same direction. Such

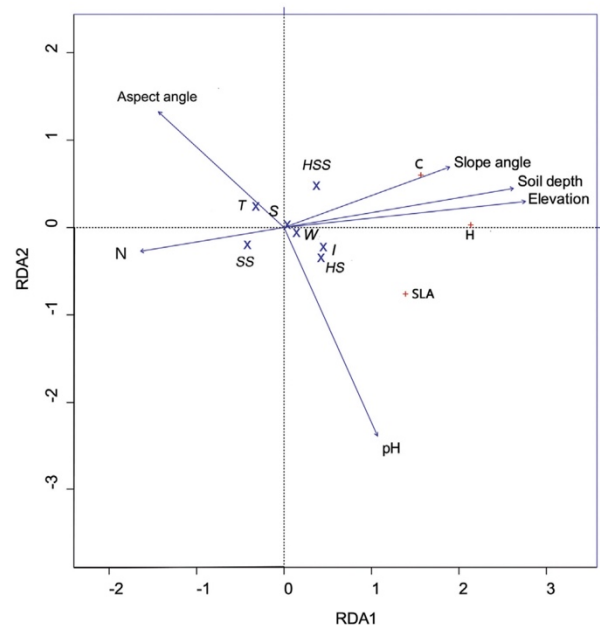


Fig. 1. Redundancy analysis ordination graph (scaling 2) for *Brachypodium genuense* data (SLA – specific leaf area; H – mean plant height; C – plant cover percentage), using elevation, aspect angle, slope angle, landform, soil depth, pH and nitrogen concentration (N) as explanatory variables (HS – hollow surface on slope; HSS – hollow on semi-flat surface; I – “impluvium”, concave water drainage surface; S – slope; SS – semi-flat surface; T – “tread”, flat or semi-flat part of a step in a terraced slope; W – convex surface straddling a watershed).

results also indicate that a higher competitive ability (resulting in a high tussock horizontal and vertical space occupation) reflected in a high SLA value. On the other hand, aspect, pH, soil depth and the joint effects of landform and elevation and of nitrogen and soil depth emerged as the main drivers of the trait–environment relationship. In particular, the higher values of the considered traits and tussocks dimension were related to deep soils and conservative landforms (impluvium and hollow surface on slope). It has been observed that high soil depth and/or conservative landforms underlie highly productive conditions, since they increase the soil water holding capacity (Franzluebbers, 2002), thus reducing the summer drought stress typical of the sub-Mediterranean climate, where even small context-dependent variations can lead to substantial changes in site ecology (Catorci et al., 2014c). Conversely, on “tread” landform, leaching might prevent nutrient accumulation, while shallow soils determine low water availability, reflecting in populations with lower values of SLA, plant stature and cover value of tussocks. These findings are consistent with previous observa-

Table 2

Results of partial redundancy analyses performed using specific leaf area, plant height and cover percentage of *Brachypodium genuense* as response variables, constrained by topographic and edaphic variables, separately. Both single and joint effects (*adj. R*²) of the two groups of variables are shown. For the joint fractions, *adj. R*² values greater than 0.01 are reported.

| | Topographic variable | <i>Adj. R</i> ² | Edaphic variable | <i>Adj. R</i> ² |
|---------------|----------------------------------------------------|----------------------------|-------------------------|----------------------------|
| Single effect | Elevation | 0.0144** | pH | 0.0622*** |
| | Aspect angle | 0.0122* | Nitrogen | 0.0006 ^{n.s.} |
| | Slope angle | 0.0056 ^{n.s.} | Soil depth | 0.1109*** |
| | Landform | 0.0543*** | | |
| Joint effect | Landform and elevation | 0.0691 | Nitrogen and soil depth | 0.0626 |
| | Landform, slope angle and elevation | 0.0385 | | |
| | Elevation, aspect angle, slope angle, and landform | 0.0156 | | |
| Residuals | | 0.7628 | | 0.7924 |

^{n.s.}: not significant.

*** *P* = 0.001.

** *P* < 0.01.

* *P* < 0.05.

Table 3

Scores of the topographic and edaphic variables on the first three axes of the redundancy analysis ordination graph (scaling 2) obtained by processing the matrix “relevés × *Brachypodium genuense* traits (specific leaf area, plant height and cover percentage)” constrained by topographic and edaphic variables.

| Explanatory variable | Scores | | |
|---------------------------------------|----------|----------|----------|
| | Axis 1 | Axis 2 | Axis 3 |
| Elevation | 0.70236 | 0.07504 | 0.14941 |
| Aspect angle | -0.36386 | 0.33616 | -0.52244 |
| Slope angle | 0.48202 | 0.17511 | 0.08624 |
| Landform: “watershed” | 0.11913 | -0.05041 | 0.35593 |
| Landform: “impluvium” | 0.30983 | -0.16467 | 0.42662 |
| Landform: semi-flat surface | -0.42725 | -0.17142 | 0.22965 |
| Landform: hollow surface on slope | 0.36837 | -0.32605 | -0.24656 |
| Landform: “tread” on a terraced slope | -0.41641 | 0.28216 | 0.39857 |
| Landform: slope | -0.00722 | 0.10379 | -0.65241 |
| Soil depth | 0.66790 | 0.11370 | -0.05891 |
| Soil pH | 0.27164 | -0.60614 | -0.01884 |
| Soil total nitrogen content | -0.41643 | -0.06998 | 0.11118 |

tions on the relationship among leaf traits/plant height and habitat productivity (Bernard-Verdier et al., 2012; Laliberté et al., 2012). Moreover, they support Grime’s statement (2001) that productive habitats are more suitable for dominant tall grasses, and confirm the high competitive ability of *B. genuense* in productive conditions (Corazza et al., 2016).

Overall, variance explained by the considered environmental variables was 34.1%; this means that other variables may play a key role in the variation of the considered traits. In fact, trait values could change due to patch disturbance history and the effect of leaf age (Laliberté et al., 2012; Dwyer et al., 2014). Moreover, each tussock could be affected by the performances of other tussocks, connected by long-ramets and living in different micro-environmental conditions (De Kroon and Knops, 1990). Finally, other chemical and physical features of the soil that were not considered in the present study may affect trait variation and cover values.

In general, we can confirm the suggestions (e.g. Laliberté et al., 2012; Dwyer et al., 2014) asserting that environmental factors can have interactive effects on SLA values and that traits vary within species in different ways depending on the considered gradient and reflecting the different contribution (positive or negative) of each factor on the site’s productivity.

4.2. Specific relationship between environmental parameters and traits

4.2.1. Aspect and slope angle

Concerning the interplay between SLA/plant height/cover values and the environmental parameters considered singly, we found higher values on north-facing slopes than on the south-facing ones. Our results are in agreement with the findings of Wellstein et al. (2013), who referred to four grassland species, among them *Sesle-*

ria nitida, another sub-Mediterranean tall grass. It was argued that low SLA enhances the tolerance to water and nutrient shortage (Fonseca et al., 2000), contributes to limit leaf transpiration and improves plant efficiency in using high light intensities (Gratani et al., 2014). The conservative strategy fostered by low SLA (Wright and Westoby, 2002) gave rise to low plant height and cover value of tussocks, especially on south-facing slopes. In fact, the greater radiation on these slopes in the summer dramatically reduces the soil water content (Joffre and Rambal, 1993). Drought stress, in turn, reduces soil nitrogen availability, lowering microbial activity (Larsen et al., 2011), thus decreasing the plant nitrogen uptake (He and Dijkstra, 2014) and limiting plant development. Although we observed a wide range of slope angle variations (1–70°), slope angle singly considered did not significantly affect trait and cover values (Table 2), likely because it varied jointly with other more effective variables (especially elevation, soil depth, and landform, see Fig. 1).

4.2.2. Altitude and landforms

The observed increase of SLA and plant height values with elevation seems in contrast with some previous studies. In fact, Kichenin et al. (2013) found an intra-specific decrease of SLA and plant height values with increasing elevation. However, this research focused on an elevation gradient inside alpine and subalpine climates, where the stress increased toward higher elevations, characterized by lower air temperature, lower partial pressure of CO₂, higher UV radiation (Körner, 2007), and lower nutrient availability (Huber et al., 2007). Our findings, instead, were in accordance with Gross et al. (2013), who found a similar pattern in the Mediterranean climate, even if at inter-specific level. In the Mediterranean-type climate, water availability decreases with decreasing elevation, while temperature shows the opposite trend, generating an aridity stress at low elevations (Stultz et al., 2007). In this way, as

observed by Bernard-Verdier et al. (2012) at the inter-specific level for perennial herbs, it seems conceivable that individuals of *B. genuense* with a conservative and stress-tolerant strategy (small plants with low SLA) were found at lower elevation and on shallow soils. Instead, at higher elevation and in productive conditions, individuals tend to show acquisitive strategies related to higher SLA and plant height, leading to asymmetrical competitive exclusion of subordinate species (Bernard-Verdier et al., 2012). It was also argued that high elevations can foster high SLA values since thin and broad leaves increase the efficiency of light acquisition, helping plants to better overcome the short growing season (Gonzalo-Turpin and Hazard, 2009), increasing their relative growth rate in the pre-reproductive phase and converting the acquired resources into seeds before the end of the growing season (Garnier, 1992). The results of our study case seem to support these statements: we observed that the combination of elevation and landform explains a significant portion of the constrained trait/cover variability (Table 2) and that at the higher elevations, conservative landforms are of greater importance (Fig. 1). In fact, these conditions imply long-lasting snow cover; indeed, it has been demonstrated that species growing in late snowmelt locations exhibit higher SLA values (Baptist et al., 2010), since this may allow plants to optimize carbon gain by producing low-cost leaves and therefore to overcome the constraints of a short growing season (Coholer, 2005).

4.2.3. Soil features

We found a positive correlation between trait/cover values and soil pH, in that acid soil gives rise to low SLA, plant height and cover values. In fact, high acidity (pH below 5.0) acts in limiting root penetration and proliferation (Doss and Lund, 1975) and excess of H^+ ions may lower the root absorption of other cations (Fawzy et al., 1954), limiting the ability of plants to grow (Foy, 1992). In the study case, soil pH ranges from mean values around 4.0 to values close to 7.0, that is, from unproductive to productive conditions (Cavieres et al., 2007). This makes the observed correlation with pH consistent from an ecological point of view with the responses obtained for the other parameters considered.

The negative relationship with soil nitrogen content was a quite surprising result. In fact, several studies found that increasing nitrogen availability leads to an increase of dominant tall grasses biomass (Holub et al., 2012) and SLA values (Laliberté et al., 2012; Dwyer et al., 2014). Moreover, Holub et al. (2012) and Bonanomi et al. (2013) stated that an increase in nitrogen concentration provides a competitive advantage to perennial tall grasses. However, the lowest recorded nitrogen concentration in the present study was around 5 g kg^{-1} , which is a quite high nitrogen amount (Giardini, 2002). It was stated that high nitrogen soil content slows plant growth, increases water consumption (Skeffington and Wilson, 1988) and negatively interacts with absorption of other essential soil elements, such as phosphorus (Mohren et al., 1986). In fact, phosphorus limitation proved to reduce intra- and inter-specific SLA values (Laliberté et al., 2012; Dwyer et al., 2014). It should also be noted that in sites with low pH, a high organic nitrogen fraction, not directly available to plants, accumulates in the soil because of the low efficiency in organic matter mineralization. Thus, our findings could partly be a by-product of very low soil pH values.

4.3. Intra-specific SLA variation along productivity gradients

The median value of SLA ($17.4 \text{ mm}^2 \text{ mg}^{-1}$) positioned *B. genuense* between the slow- and the fast-growing species. In fact, Rosbakh et al. (2015) found that fast-growing performance was related to species with SLA values of about $30 \text{ mm}^2 \text{ mg}^{-1}$, while species with slow-growing strategies have SLA values of about

$10 \text{ mm}^2 \text{ mg}^{-1}$. However, we found a marked intra-specific SLA variation (from 8.9 to $36.4 \text{ mm}^2 \text{ mg}^{-1}$), thus indicating the ability of *B. genuense* to modulate from a slow- to a fast-growing strategy (Wilson et al., 1999). Consequently, we could infer that the invasive/dominant behavior of competitive-stress tolerant tall grasses (*sensu* Grime, 2001) is related not only to the clonal integration strategy, plant height, litter deposition, etc. (Canals et al., 2017), but also to the morphological variability of leaves, which allows plants to maintain the coordination of the acquisition of multiple resources and, hence, to sustain dominance in a wide range of environmental conditions.

5. Conclusion

Our results reveal that the ecological strategy of *Brachypodium genuense* is driven by environmental conditions that strongly affect its performance across a drought/productivity gradient. In fact, in productive conditions (deeper soil, medium/high pH values, north-facing slopes, “impluvium” and “hollow surface on slope” landforms), individuals of *B. genuense* showed higher SLA, plant height and cover values than in harsh conditions (south-facing slopes, shallow soils, very low pH values, “tread” landform). This highlights a fast-growing strategy as well as high competitive ability on productive sites. We cannot say how much of the observed intra-specific variation was due to phenotypic plasticity or to genetic differences. However, as inferred by Dwyer et al. (2014), given the local scale of our study and the well-demonstrated plasticity of herbs in response to environmental variations, it is likely that phenotypic plasticity may play a role in this system.

Our findings also indicated that *B. genuense* invasion might be hampered by very high levels of nitrogen (and low soil pH) and by high soil water shortage. These findings, besides increasing our knowledge on the ecology of *B. genuense*, provide some guidance for the planning of management actions, since they can help to identify sites more prone to *Brachypodium* invasion and, therefore, areas where to intensify efforts of treatment through the use of herbivores or hay mowing.

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Belowground organ dry matter content: contrasting patterns of trait variation at species and community level

Alessandro Bricca¹, Gianluigi Ottaviani², Lars Götzenberger², Francesco de Bello², Maurizio Cutini¹, Jitka Klimešová²

Affiliation

¹Department of Science, University of Roma Tre, Rome, Italy;

²Institute of Botany, Czech Academy of Sciences, Dukelská 135, 37982 Třeboň, Czech Republic

Introduction

Predicting plant responses to changing environments has long been a primary topic in ecology (Schimper, 1903; Whittaker, 1960). Linking species and community responses across environmental gradients can facilitate inferences about effects caused by variations in abiotic and biotic conditions on plants (Cornwell & Ackerly, 2009; Dwyer et al., 2014; Vojtkó et al., 2017). This task can be effectively achieved by investigating functional traits, i.e. any eco-physiological and morphological features informing on plant fitness (Lavorel & Garnier, 2002; Violle et al., 2007). In recent years, the number of studies using easy-to-collect functional traits have increased (Garnier et al., 2016; Diaz et al., 2016; Funk et al., 2017), also fostered by the accumulation of plant trait data in global databases (e.g. TRY, Kattge et al., 2011). However, we are still far away from having a precise scheme of plant functional strategy that allow the prediction of plant distribution along environmental gradient. Indeed relevant functional dimensions are poorly assessed. Firstly, a large part of plant traits' variation, for example belowground functional space, remains still largely unexplored (Shipley et al., 2016; Klimešová et al., 2018). Secondly, the role of intraspecific trait variation in community studies is often neglected (Lepš et al., 2011; Volf et al., 2016).

Although belowground plant ecology research is experiencing a spiking interest, advancements are mainly focused on traits related to fine roots and mycorrhizal associations reflecting only root acquisition strategy (Laughlin et al. 2010; Kramer-Walter et al., 2016; Laliberté, 2017). Recently, ecologists highlighted other belowground important traits capturing different functions (Shipley & Vu, 2002; de Bello et al., 2012; Klimešová et al., 2018). However, studies addressing the belowground functional traits and strategies of plants, compared to their aboveground counterparts, remain poorly investigated (Laliberté, 2017). This limitation is mainly due to the laborious and time consuming field work as well as to the lack of standardized procedures that would be required for collecting and measuring samples consistently across different ecosystems (Holdaway et al., 2011). Belowground traits that are easy to collect, capturing different functions than acquisition, are therefore urgently needed. As a candidate for such straightforward belowground trait, we propose belowground organ dry matter content (hereafter BODMC). Similar traits to BODMC have been examined, such as root dry matter content (Shipley & Vu, 2002) or belowground organ water content (de Bello

et al., 2012). However, we consider any belowground organ (e.g. root, rhizome, bulb, tuber) and we express the trait value with the same units of measurement of the aboveground counterpart, that is, leaf dry matter content (hereafter LDMC; Perez-Harguindeguy et al., 2013). This trait was used only several times as a functional trait so, that we have only limited information about its correlation with other traits and with environmental conditions. It seems to be negatively correlated with leaf dry matter content, plant height and with moisture availability (Shipley & Vu, 2002; de Bello et al., 2012). We can expect that contrary to LDMC, BODMC does not reflect only content of structural tissue in organ (e.g. Kazakou et al., 2006) but also content of storage carbohydrates (Janeček et al., 2011). We suggest that BODMC can capture an independent axis of plant performance related to belowground resource conservation strategy, which in turn affects plant ability to persist under given environmental conditions.

Relationship of functional trait to environmental gradient in field cannot be simply studied only on species level since some effects were unable to quantify. Species are indeed assembled and organized into communities in which they occur generally with different abundances. Even if any trait information is unavoidably gathered at the level of species (Violle et al., 2007), neglecting the abundances of the species may lead to misleading interpretations. Indeed, it has been seen that most abundances species affect strongly the trait-environmental relationship (Cingolani et al., 2007; Muscarella & Uriarte, 2016) as well as a key effect on the ecosystem functioning (Grime, 1998). Therefore, scaling trait information from species to community level allow a better understanding of plant-environment relationships (Borgy et al., 2017). Usual way how to study functional composition on community level is throughout the community weighted mean (hereafter CWM; Garnier et al., 2004) and trait dispersion, i.e. how much co-existing species are functionally dissimilar (Villegger et al., 2008; Mason et al., 2005; Laliberté & Legendre, 2010). CWM reflects the trait-environmental relationship quantifying shift in the mean trait values due to environmental selection for the given functional trait. Community trait variation may be caused by intraspecific trait variation (hereafter ITV), by variation in species composition, and by the covariation of ITV and species composition effects (Lepš et al., 2011). The relative contribution of each effect remain largely undetected with trait retrieved from database, since neglecting ITV has usually result in underestimation of community trait composition response to environmental changes. Thus, predicting plant community responses to environmental changes requires disentangling the relative contribute of inter- and intraspecific trait values and their combined effects (Lepš et al., 2011; Violle et al., 2012; Volf et al., 2016). As regard trait dispersion, community characterized by a higher dispersion (i.e. trait divergence) highlighted co-existence of species with a wider variety of functional strategy (i.e. niche differentiation), compared to community characterized by lower dispersion (i.e. trait convergence) in which the species that coexist are functionally similar (i.e. niche overlapping due to e.g. biotic and abiotic filtering; Mason et al., 2011; de Bello et al., 2013a). Different traits usually contribute to species coexistence in community differently, as was found for different aboveground traits (Grime, 2006; Spasojevic & Suding, 2012). We can expect that this holds especially true when considering aboveground versus belowground traits. Indeed, even if it has been suggested that belowground functional traits may show opposite pattern to

aboveground (Mayfield & Levine, 2010; Spasojevic & Suding, 2012), there are few evidences supporting this assumption (Vojtkó et al., 2017).

In consideration of the above, this research sets out to analyze relationship between aboveground and belowground functional traits at species and community level along a gradient of environmental constrain, as well as the contribute of inter- and intraspecific trait variability at community level along the same environmental gradient. We hypothesized a different relationship between aboveground and belowground functional traits on species and community level, and that community functional trait variation along the gradient will be explained by a large part of intraspecific trait variation. Specifically, our questions are: 1) Do dry matter content of belowground organs represent an independent axis of plant ecological strategy, i.e. storage of water and nutrients, in comparison to LHS scheme and, more specifically, in relation to leaf dry matter content?; 2) What is the relationship between functional dispersion for dry matter content of aboveground and belowground organs?; 3) Are changes in community weighted means of the two traits associated with resource use and storage efficiency (BODMC and LDMC) along the elevation gradient more affected by intraspecific variability or by changes in species composition?

METHODS

Study Area

The study area is Mt. Velino Massif (2486 m a.s.l.), located in the Central Apennine (Abruzzo region; Fig.1), Italy. The limestone massif (Petriccione 1993) is characterized by a sub-Mediterranean climate defined by a summer drought period and cold-stress during the winters occurred with different intensity and timespan at different part of the elevation gradient. Five termopluviometric station, placed along elevation gradient at regular intervals of 250 m starting at 1200 m, measured precipitations, air and soil temperatures from 2006 (Appendix 1). In the growing season (May-September), the mean temperatures are around 17°C both for air and soil and precipitation are 350 mm at 1200 m a.s.l. while at 2200 m they are around 8°C, 0°C and 470 mm (Theurillat et al., 2011, Theurillat et al., unpublished data). During the winter season (November-February) the mean temperatures for air and soil are 3.32°C and 4.29°C at 1200 m a.s.l., while at 2200 m a.s.l. is around -3.46°C and 0.24°C (Table 1). The area in the past has been almost totally deforested and secondary dry grasslands, dominated by *Sesleria juncifolia*, *Globularia meridionalis*, *Carex humilis*, *Bromus erectus* are widespread from the lowest part of the area (ca. 1250 m a.s.l.), up to ca. 2000 m a.s.l. Above 2000 m a.s.l., the secondary grassland was replaced by primary grassland and the vegetation is going to shift towards cryophilous species (e.g., *Carex kitaibeliana*, *Silene acaulis* and *Potentilla crantzii*) (Petriccione, 1993).

| Termopluviometric station | 1 | 2 | 3 | 4 |
|---------------------------|--------|--------|--------|--------|
| Altitude (m a.s.l.) | 1450 | 1700 | 1950 | 2200 |
| T (°C) | 15.02 | 12.92 | 11.47 | 7.79 |
| T winter (°C) | 2.17 | 0.06 | -0.40 | -3.46 |
| MAT | 8.64 | 6.52 | 5.50 | 2.06 |
| P (mm) | 434.28 | 419.07 | 456.36 | 455.64 |
| PET (mm) | 89.53 | 85.40 | 78.86 | 72.17 |
| Aridity Index (P/PET) | 1.07 | 1.09 | 1.31 | 1.53 |

Table 1. Climatic characterization of the study area. Mean values of air temperature (T), sum annual precipitations (P), potential evapotranspiration (PET), aridity index (Middleton and Thomas 1992) during the growing season (May to September). T winter = mean values of air temperature during the winter season from November to February; MAT = mean annual temperature.

Vegetation and Traits sampling

We based our study on an existing dataset containing 84 permanent plots (Theurillat et al., 2011). In order to maximize the homogenization of the environmental conditions across plots along the elevation gradient, i.e., to better detect the role of elevation on trait patterns, we selected plots as follows: 1) calcareous grasslands; 2) absence of domestic grazing; 3) south-west aspect; 4) approximately constant slope. This resulted in 45 selected plots (2x2 m). In each plot, we visually estimated species cover during the 2016 growing and flowering season. Prior to the analysis, species cover records were transformed to percentage values (as mean values of the cover interval): +: 0.1%, **1a**: 2.5%, **1b**: 5% **2a**: 10%, **2b**: 20%, **3a**: 31.25%, **3b**: 43.75% **4a**: 56.25%, **4b**: 68.75%, **5a**: 81.25%; **5b**: 93.75%. As measuring traits for all species in each plot was infeasible, we focused on the most abundant species as the trait value of dominants is expected to largely affects community assembly and functioning (“mass ratio” hypothesis; Grime, 1998). Therefore, we divided the elevation range into sections of ca. 250 m a.s.l. leading to four discrete elevation belts: 1325-1575 m a.s.l. with 9 plots, 10 plots at 1575-1825 m a.s.l., 11 plots at 1825-2075 m a.s.l. belts and 15 plots at 2075-2375 m a.s.l. (Fig.1). We followed guidelines proposed by Pakeman & Quested (2007), but rather than using species’ plot cover values, we pooled all the plots in each elevation belt together. We selected all those species whose relative cumulative cover, starting with species showing high cover values, reached 80% of the total vegetation cover in each elevation belt. In total, we identified 26 species, 13 of which were spread across more than one elevation belt. Those species occurring in more than one elevation belt have been sampled for traits in each belt, e.g., *Sesleria juncifolia* found in all across the gradient gathered 4 mean traits’ values. This resulted in 50 mean trait values (averaged from 10 individuals per species per belt; see below) partitioned into 11 values for the first section,

10 for the second section, 12 for the third section and 17 for the last section. We then collected aboveground traits related to LHS scheme (SLA, plant height, seed mass; Westoby, 1998). We measured 10 leaves of 10 different individuals of each species for SLA (mm^2/mg), 15 different individuals for plant height and at least 2 seeds in no less than 3 individuals in the middle point of each elevation belt, that is near each termopluiometric station, in similar topographic conditions (slope, aspects and land form) as those of the vegetation plots (Bricca et al., unpublished).

To investigate the presence of independent belowground water conservation strategy axis of species specialization in comparison to aboveground traits, during the 2017 growing and flowering season, we carried out a second sampling of two additional functional traits (LDMC and BODMC) for the same species whose LHS traits were collected in 2016. We measured LDMC (mg g^{-1}) in 10 healthy, fully expanded leaves from 10 different individuals per species per elevation belt, following standardized protocols (Pérez-Harguindeguy et al., 2013). For BODMC, we decided the number of individuals to sample depending on the field effort to collect the belowground organ(s): we generally excavated 4 individuals per species per belt, while we gathered two individuals per belt for those species that required more time to be dug up (Appendix 2). BODMC was calculated as per LDMC, that is, the dry weight of the belowground organ (mg) divided by its fresh weight (g). Similarly to the LHS traits, we sampled all the individuals in the middle point of each elevation belts having comparable environmental condition (topographic features: slope, aspect and land form) as those of permanent plots. For BODMC, in the field we dug up whole plant and we kept the plant material in plastic bags having the belowground organs maintained in wet conditions by wrapping the organ with a moist paper tissue. Then, the plastic bags were stored in freezer and the fresh weight of belowground organs was measured within 24 h since sample collection. Once in laboratory, we cut and selected the oldest parts of the belowground organs, avoiding to collecting younger parts that are more related to acquisitive functions (e.g., bearing fine roots), as BODMC is related to resource (e.g., water) conservation. Additionally, the samples were oven-dried at 60°C for approximately 96 h, i.e., until they were completely dry.

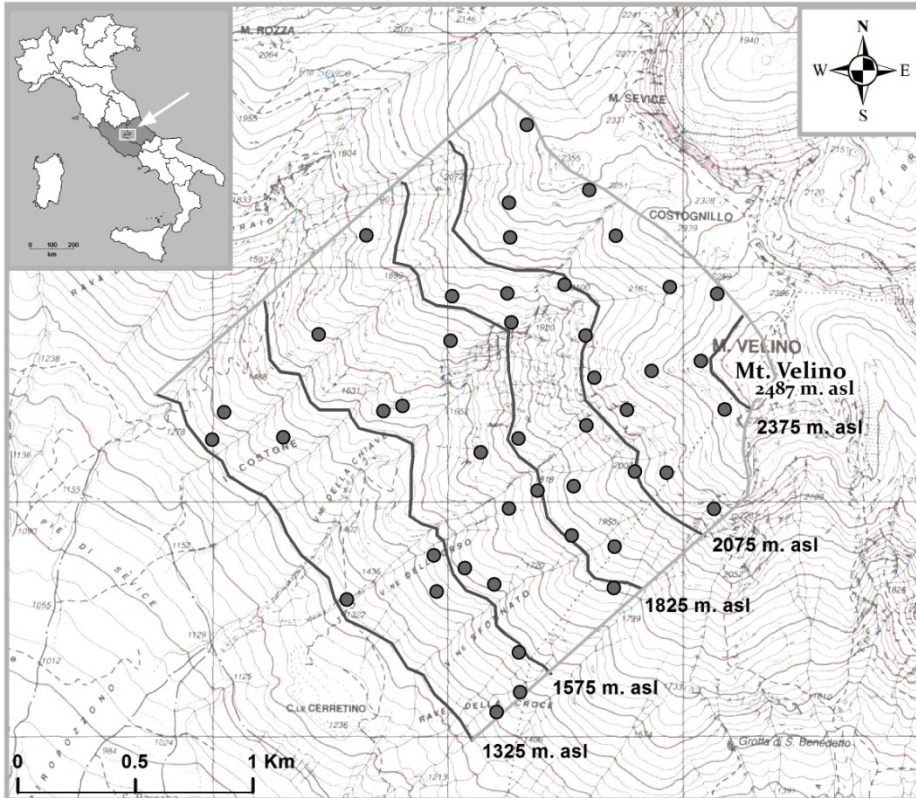


Fig.1. Location of the study area in Italy (Mt Velino Massif, Central Apennines; rectangular inset). Continuous lines represent the elevation section, and dots are the 45 permanent plots selected for traits sampling.

Statistical analysis

Coordination among traits

We performed principal component analysis (PCA) to investigate relationships among aboveground (i.e., SLA, plant height, seed mass, LDMC) and belowground (i.e., BODMC) traits, using species mean trait values. Before computing the PCA, all traits have been standardized. We used the function *rda* for computing the PCA and the function *scale* to standardize the variables in the *vegan* package.

Functional structure metrics

We examined functional patterns using the community weighted mean (CWM; Garnier et al., 2004) and functional dispersion (FD_{is}; Laliberté & Legendre, 2010). We computed the indices for each permanent plot using species cover at plot scale and trait values collected at the mid-point of each elevation belt (see Vegetation and Traits samplings). We calculated the community-weighted means for both traits, LDMC and BODMC (hereafter CWM_{LDMC}, CWM_{BODMC}) with the following equation (Garnier et al., 2004):

$$CWM = \sum_{i=1}^n p_i x_i$$

where *CWM* is the community-weighted mean value of a given functional trait, p_i is the relative abundance of the *i*-th species and x_i is the mean trait value of the species *i*. Disentangling the effects of ITV and species

composition on community functional structure required the calculation of two different CWM, the so-called “specific” and “fixed” CWM. Specific CWM were calculated using the trait value recorded at belt level (the averages of all individuals of a species found in the belt). Fixed CWM instead, were computed using the mean trait values across all belts in which the species has been found.

We calculated functional dispersion (FDis; Laliberté & Legendre, 2010) as index of trait dispersion. FDis represent the mean distance of individual species to the centroid of all species in the community and it is expressed with the following equation:

$$FDis = \frac{\sum a_j z_j}{\sum a_j}$$

where a_j is the abundance of species j and z_j is the distance of species j to the weighted centroid (Laliberté & Legendre, 2010). We selected the FDis index because has several advantages over other dissimilarity indices: it is not strongly influenced by outliers, second it is unaffected by species richness (Laliberté & Legendre, 2010). High/low values of FDis In computing FDis index for LDMC and BODMC (hereafter FDis_{LDMC}, FDis_{BODMC}), we considered gower distance as measure of traits dissimilarity (Pavoine et al., 2009).

All indices (Specific and Fixed CWMs as well as FDis) were computed with *dbFD* function in the R (2017) *FD* package version 1.0 (Laliberté et al., 2014).

Modelling functional patterns across the elevation gradient

Elevation (m a.s.l.), a proxy for macroclimate conditions (Bassler et al., 2016), was used as predictor and CWM_{LDMC}, CWM_{BODMC}, FDis_{LDMC} and FDis_{BODMC} as response variables in individual models. As the assumption of residuals’ homoscedasticity was not met, we performed generalized least-square models (GLSs) using the *nlme* package 3.1 (*gls* function), choosing the most appropriate variance structure according to the minimum AIC (Zuur et al., 2009; Bitomsky et al., 2018). The CWMs for both traits, were log-transformed before running the GLSs and improve the normality of the residuals

To decompose the effect of ITV and species turnover on CWM we followed the approach of Lepš et al., (2011). This method is based on the decomposition of the total sum of squares of the plot-level trait variance related to an environmental variable (in our case, elevation) into “fixed”, “intraspecific” and “covariation” effects, that is, $SS_{\text{specific}} = SS_{\text{fixed}} + SS_{\text{ITV}} + SS_{\text{cov}}$, in a repeated measures ANOVA. To the best of our knowledge we are not aware of any other method allowing to decompose the effects of ITV and species composition. To run ANOVA we used the function *trait_flex_anova* in Supplementary Materials provide by Lepš et al., (2011). As for GLSs, also the “specific” and “fixed” CWMs for both traits, were log-transformed before running the ANOVAs to improve the normality of the residuals.

Since vegetation on long environmental gradient generally may response with unimodal variation (Lepš & Šmilauer, 2003), we accounted also for non-linear relationship for our analysis introducing a quadratic term in each of the above-mentioned regression model.

Results

Aboveground and belowground traits coordination

We identified three independent axes of specialization explaining together 81% of the total variation of the five traits (Fig. 2a, b).

Plant height, SLA and BODMC are traits unequivocally correlated with each of the PC axis (Fig. 2, a-b). In detail, we found that the variance explained by the first axis (PC1) is 34% with tall species found at the positive end of the axis. The second axis (PC2) explained 28% of the total variance with species found at the positive end of PC2 axis showed lower SLA values. The third axis (PC3) finally explained 19% of the total variance (Fig. 2, b) and it was positively associated with BODMC values. Seed mass and leaf dry matter content were associated with a couple of axes. Indeed, seed mass resulted related positively with the first axis and negatively the second, leaf dry matter content, instead, resulted positively associated both to the first axis and the second axis as well. Traits PCA scores are reported in Appendix 3.

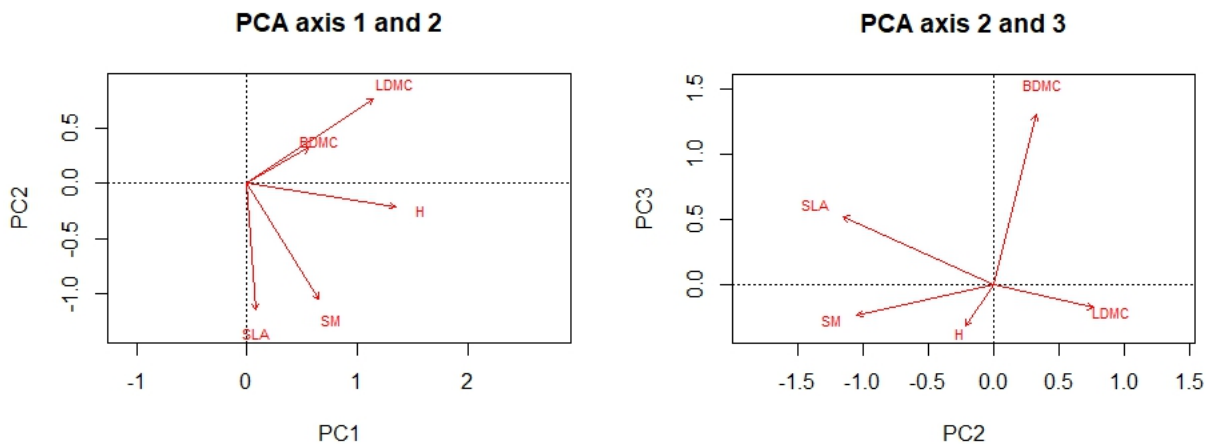


Fig.2 Principal component analysis (PCA) of mean species traits of the study site (Mt Velino grasslands).

Aboveground and belowground dry matter content community variation along environmental gradient

We found that the best fitting model for FDi_{SLDMC} index highlighted a unimodal relationship with the elevation: lower values at both end of the gradient and higher values at intermediate elevations (slope value= - 0.14; p-value = 0.001; Fig.3,c). We found no significant relationship between CWM_{LDMC} and elevation.

For BODMC, both CWM and FDis showed unimodal relationship with elevation. CWM_{BODMC} highlighted lower values at both ends of the elevation gradient and a peak at intermediate belts (slope value= -0.19; p-value= 0.008; Fig.3,c), while FDi_{BODMC} showed higher values at both end of the elevation gradient and lower values at intermediate elevations (slope value: 0.15; p-value< 0.001; Fig.3,a). Further information on GLS models are reported in Appendix 4.

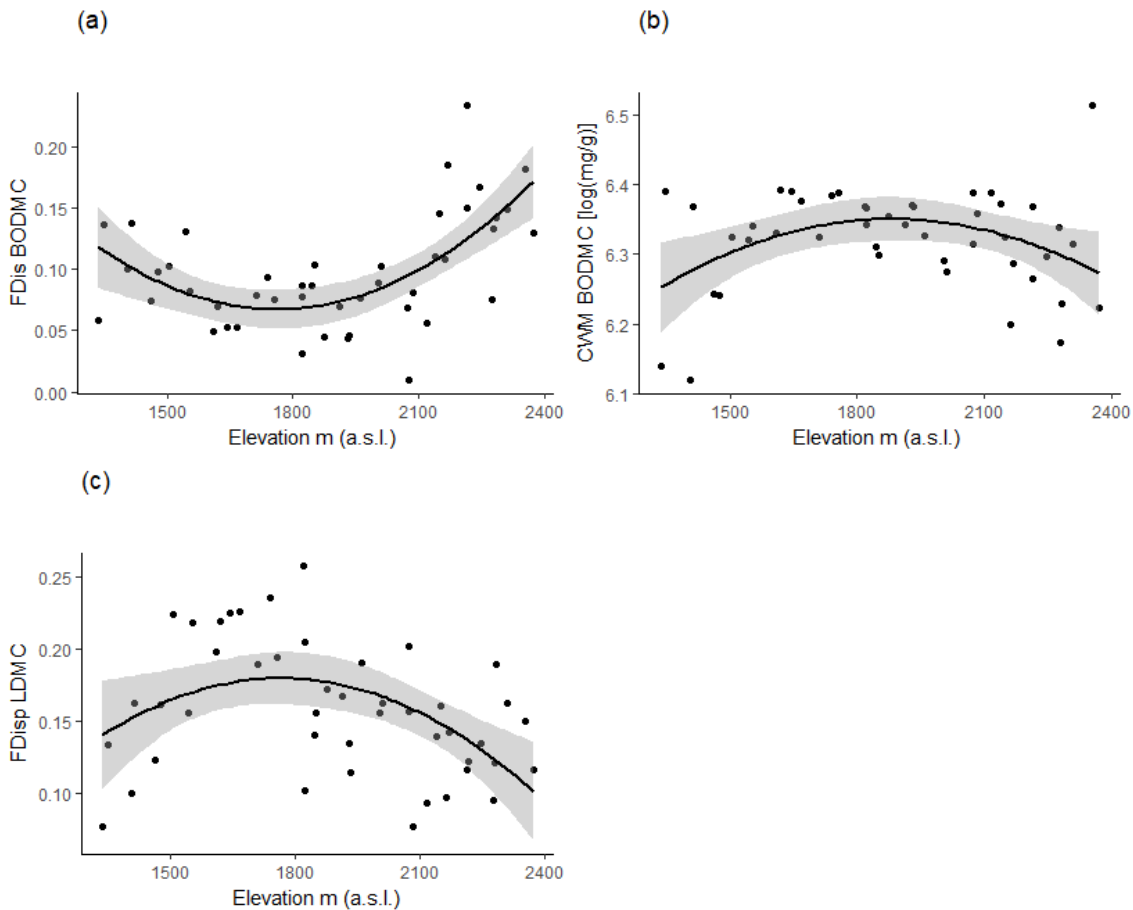


Fig 3. Generalized least square models (GLSs) of above- and belowground dry matter content traits (LDMC, BODMC) along elevation gradient on Mt Velino grasslands. Left panels (a, c) refer to patterns of functional dispersion, while right panel (b) illustrates changes of CWM with elevation.

The analysis of decomposing ITV and species composition effects on trait variability without any environmental variables, revealed an almost equal contribution of both effects on BODMC and LDMC as well, with a negative covariation (Table 2). Considering how much is the proportion of variation explained by the elevation for each of the effects, we found that for BODMC elevation explained 14% of the total variance of the community trait variation, partitioned in 9% for ITV, 1% due to species composition and 3% for the positive covariation. The effect of the elevation resulted non significant only for the species composition. Regarding the effect of elevation on LDMC community variation, we found that the model explained 1% of the total variance, partitioned in 18% of ITV, 10% for species composition and -27% due to negative covariation (Table 2).

| | | BODMC | LDMC |
|-----------|-------------|-------|---------|
| Total | Turnover | 0.63 | 0.7 |
| | ITV | 0.58 | 0.5 |
| Elevation | Turnover | 0.01 | 0.1* |
| | ITV | 0.09* | 0.18*** |
| | Covariation | 0.03 | -0.27 |
| | Total | 0.14* | 0.11 |

Table 2. Results of quadratic ANOVAs for BODMC and LDMC. It is reported 1) the contribution of species turnover and ITV to total variation; and 2) how much of the total variation, species turnover, ITV and covariation is explained by the elevation. (*:p<0.05; **: p<0.01; ***:p<0.001).

Discussion

Aboveground versus belowground plant strategies

We predicted a tradeoff between aboveground and belowground traits related to resource acquisition, use and storage. One of our key results was the lack of correlation between BODMC and any aboveground functional traits, in particular with LDMC (Fig. 2). Studies dealing with this topic were focused only on acquisitive resource rate (i.e., fine root traits; de la Riva et al. 2017), findings which propose belowground resource acquisition as a new functional dimension in the plant economic spectrum (Kramer-Walter et al., 2016). We revealed that BODMC might play an important, independent role determining the plant performance as it explained a considerable amount of the total variation (19%). The lack of trade-off between aboveground and belowground dry matter content could be due to the fact that BODMC incorporates structural tissue and also storage carbohydrates, representing the reliable proxy of resource conservation.

The fundamental functions of leaves is resource acquisition (i.e. via photosynthesis; Laliberté, 2017) implying the regulation of gas exchanges and evapotranspiration through controlling of stomatal activity and tissue density (Wright et al., 2004; Laughlin et al., 2010). Succulent leaves could be the exception of this general rules (D'Antonio, 1993). However, these species with these were absent in our case. LDMC also largely influences leaf lifespan (positively) and relative growth rate (negatively; Wright et al., 2004), hence resource conservation is a secondary function. BODMC, on the other hand, is highly associated with resource conservation and storage, as belowground resource acquisition is carried out by fine roots or mycorrhizal associations (Laliberté, 2017). Indeed, the ability of the perennial or biennial plant to persist in the soil was generally associated to the ability to produce new shoot from the belowground organs (e.g. rhizomes, bulb,

main root) after unfavorable season or a disturbance regime. This could be effectively achieved by allocation resource in the belowground organ (Klimeš & Klimešová, 2002).

The negative relationship between leaf traits was in line with our expectation since different works found the same association (de Bello et al., 2012; Perez-Harguindeguy et al., 2013), that represented the fundamental evidence on which the “leaf economic spectrum” was built on (Wright et al., 2004): leaves were constrained by long lifespan with short growth rate and by short lifespan with high growth rate. The lack of any coordination between plant height and SLA was in line with previous suggestion (Westoby, 1998), assessing that SLA and plant height capture different aboveground functions, i.e. resource acquisition and competition for light (Westoby, 1998; Diaz et al., 2016). The positive relationship between seed mass and SLA was unexpected finding (Westoby, 1998; Diaz et al., 2016). As the reproduction represent an effort for the plant (Obeso, 2002), it could be that higher resource availability allow an higher resource store in the seed.

Plants store water in aboveground organs, such as leaves and stem (Shipley and Vu, 2002). However, even if they have other primary functions, they can also jointly storage resource. Contrary, this function is well exerted by any of belowground organs (such as main root, rhizomes, tuber, bulb). Therefore, we can safely infer that BODMC is a reliable proxy for the function of resource conservation.

Patterns of functional dispersion between aboveground and belowground dry matter content

Changes in functional dispersion of LDMC and BODMC were predicted by unimodal relationship with the elevation. Interestingly, these indices showed opposite pattern with lower $FDis_{LDMC}$ values and higher $FDis_{BODMC}$ values revealed at both ends of the gradient. In this sense, our results contribute to add new evidences to previous suggestions (Mayfield and Levine, 2010; Spasojevic & Suding, 2012; Vojtko et al., 2017). In our case, this opposite pattern could be due to the effect that changing water availability has on grassland communities. Lower water availability at lower and higher elevations can be caused by the higher evapotranspiration and lower temperatures, respectively (Table 1), and may have selected lower number of strategy for $FDis_{LDMC}$, i.e. niche overlap. As it was previously suggested, species to co-exist can't show a niche completely overlapped (Gause, 1964; MacArthur & Levins, 1967). Therefore, species co-existence could be achieved by belowground niche differentiation. This suggestion could be in line with Bricca et al., (unpublished) who found for plant height, SLA and seed mass the same functional pattern of LDMC along the same elevation gradient. Under more constrained conditions, the co-existence is enhanced by the differentiation of traits that maximize acquisition and retention of limiting belowground resources (e.g. Coomes & Grub, 2000). However, further investigation at community level along environmental gradient is needed.

Community trait mean variation and relative contribution of ITV and species composition

Contrary to $FDis$ indices, we found for CWM indices a weaker relationship with the elevation gradient for BODMC and a lack of any relationship with LDMC. Previous studies have found consistent variation in CWM under different environmental gradients (Spasojevic & Suding, 2012; de Bello et al., 2013b). However, those

gradients encompassed different environmental conditions and habitats, therefore the shifts in CWM could be mainly related to species composition changes. Otherwise, our results were in line with Pescador et al., (2014), that analysing the relative contribution of ITV and species composition along an homogeneous elevation gradient in Mediterranean region, found a low variability in CWM along the elevation gradient.

In our case elevation significantly explained most of ITV variability (Table 2) for both traits. The high presence of ITV could be arise since along elevation encompassed mostly one single habitat, i.e. secondary grassland ranging from 1325 to ca. 2000 m. a.s.l. Above the 2000 and up to 2375 m. a.s.l., secondary grassland is replaced by primary grassland (Petriccione, 1993) that represent the main source of species composition effects. The interesting result was the positive covariation found for BODMC and the negative covariation (even if resulted not significant) for LDMC. The absence of an overall community response for LDMC along the elevation gradient may be due to the negative covariation (Table 2) among ITV and species turnover that compensate each other: species and individual with opposite trait value are selected.

Instead, the positive covariation highlighted a community reinforce for BODMC in response to elevation gradient: elevation selected species and individuals towards the same trait value. At lower and higher elevations, under more severe environmental conditions (i.e. summer drought and winter cold, respectively), grassland communities displayed lower belowground organ dry matter content values (i.e. more fleshy organs). Increasing the proportion of water allocated in the belowground organs could be an effective response to such harshness: being located in the soil, belowground organs are protected from higher evapotranspiration that can happen aboveground during summers, or avoiding freezing during the winters. Also, BODMC could reflect content of storage carbohydrates that may operate as osmotic protectants when plants are exposed to desiccation (Koster & Leopold, 1988) or cold (Bachmann et al., 1995). Our results therefore stress the importance of carbohydrates as key compounds for resource storage as well as osmotic protection, both enhancing plant fitness in response to dry and cold environments.

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Clonal vs leaf-height-seed (LHS) traits: which are filtered more strongly across habitats?

Anna E.-Vojtkó · Martin Freitag · Alessandro Bricca · Felipe Martello ·
Joaquín Moreno Compañ · Martin Küttim · Róbert Kun · Francesco de Bello ·
Jitka Klimešová · Lars Götzenberger

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Abstract Plant functional traits are now frequently used instead of species identity to identify how plant species co-exist in assemblages. One notion is that species inhabiting the same environment have more characteristics in common than species from different habitats, leading to different prevailing dominant traits along environmental gradients, and also to a lesser diversity of traits in habitats that impose a stronger filter on these traits. Though such patterns have been demonstrated for different environmental drivers and different traits,

studies using easily available traits connected to above ground processes (i.e. traits of the leaf-height-seed, or LHS, strategy scheme) are largely overrepresented in these analyses. Here we combined data on clonal and bud bank traits, representing the ability to reproduce and spread vegetatively, with LHS trait data and examined how these traits varied in relation to the vegetational composition of 29 Central-European habitat types. Our analysis focused on determining whether clonal/bud bank or LHS traits play an important role for

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A. E.-Vojtkó
MTA Centre for Ecological Research, Danube Research Institute,
Department of Tisza Research, Bem tér 18/C, Debrecen H-4026,
Hungary

A. E.-Vojtkó · F. de Bello
Department of Botany, Faculty of Science, University of South
Bohemia, Branišovská 31, CZ-370 05 České Budějovice,
Czech Republic

M. Freitag
Biodiversity and Ecosystem Research Group, Institute of
Landscape Ecology, University of Münster, Heisenbergstr. 2,
48149 Münster, Germany

A. Bricca
Department of Science, University of Roma Tre, V. le Marconi
446, 00144 Rome, Italy

F. Martello
Department of Environmental Sciences, Federal University of Sao
Carlos, Rodovia Washington Luis, Km 235, Sao Carlos - SP,

Brazil

J. M. Compañ
Department of Environmental Sciences and Natural Resources &
Institute of Biodiversity CIBIO, University of Alicante, Carretera
de San Vicente s/n, 03690 Alicante, San Vicente del Raspeig,
Spain

M. Küttim
Institute of Ecology, School of Natural Sciences and Health,
Tallinn University, Uus-Sadama 5, 10120 Tallinn, Estonia

R. Kun
Department of Nature Conservation and Landscape Ecology,
Faculty of Agricultural and Environmental Sciences, Szent István
University, Páter Károly utca 1, Gödöllő 2100, Hungary

F. de Bello · J. Klimešová · L. Götzenberger (✉)
Institute of Botany, Czech Academy of Sciences, Dukelská 135,
37982 Třeboň, Czech Republic
e-mail: lars.goetzenberger@gmail.com

environmental filtering along gradients approximated by Ellenberg indicator values (EIV) across these habitats. Our results show that clonal and bud bank traits are at least as – if not more – important for the differentiation of the 29 habitat types. Overall, diversity and dominance of clonal and bud bank traits was more strongly correlated with gradients of light availability, temperature, moisture, soil reaction, and nutrient availability across these habitats than it was the case for traits of the leaf-height-seed scheme. Our results call for a stronger integration of belowground traits into the functional traits approach in plant ecology and for an extension of efforts to collect such data.

Keywords community-weighted mean · Czech Republic · Ellenberg indicator values · environmental filtering · functional diversity · plant strategy

Introduction

One of the oldest questions in plant ecology tackles the processes driving species distributions in response to environmental conditions: Why are some species able to exist in particular habitats or communities, but not in others? This question remains topical today, as multiple processes have been identified to shape species coexistence, and no single mechanism accounts for all observed patterns (Vellend 2010). While species interact with both abiotic and biotic factors, it is generally agreed that environmental conditions ‘filter’ species with more suitable features (Cornwell and Ackerly 2009).

The pursuit to understand plant-environment relationships has a long history. Schimper (1898) recognized that different plant species thrive in different environmental conditions as they have features enhancing their differential fitness. More recently, such features (coined plant functional traits) have been defined as ‘the morphological, physiological and phenological features that represent ecological strategies and determine how plants respond to environmental factors’ (Pérez-Harguindeguy et al. 2013). Functional plant traits have proven to be useful tools in understanding ecological processes (e.g. Lavorel and Garnier 2002; Garnier et al. 2004), including the assembly of communities (Cornwell et al. 2006).

The effect of environmental factors on vegetation is widely recognized as ‘environmental filtering’:

Species are filtered from regional to local pools and eventually into local communities based on their traits (Díaz et al. 2004; Cornwell and Ackerly 2009; Götzenberger et al. 2012). Environmental filtering should lead to a reduction of functional diversity (FD) within communities (trait convergence – Grime 2006; de Bello et al. 2013a), because specific traits will be favoured and others selected against. For example, in alpine environments, species without the ability to survive frost will be excluded. Other traits correlated with tolerance of cold, dry conditions would also be favoured resulting in a functionally more homogenous assemblage of species in an alpine community, compared to a more general pool of species (Davis et al. 1999). Strong environmental filtering should result in co-occurring species that are more similar than expected by chance. However, biotic interactions such as competition can limit the similarity of coexisting species (MacArthur and Levins 1967). This would have the opposite effect to that of environmental filtering on trait composition resulting in increased FD (trait divergence – Mason et al. 2013; de Bello et al. 2013b). Though not mutually exclusive (Götzenberger et al. 2012; de Bello et al. 2013b), these two mechanisms are thought to predominate at different scales. At larger spatial scales (e.g. comparisons of habitats), species composition and traits are assumed to be strongly filtered by environmental factors, whereas at smaller scales (e.g. community or plot scale), species interactions and responses to disturbance are assumed to be more influential (de Bello et al. 2013b).

Most studies on plant traits and community composition focus on traits related to plant performance through competitive ability or growth capacity. The most commonly used is the leaf-height-seed (LHS) strategy scheme proposed by Westoby (Westoby 1998; Westoby et al. 2002). This scheme consists of three traits that are associated with principal biological functions: canopy height (competitive ability), seed mass (dispersal and establishment ability) and specific leaf area (rate of resource investment). However, using only LHS traits to characterize species strategies might be insufficient, as these traits do not provide information about below-ground processes (Klimešová and Herben 2015). Alternatively, clonal and bud bank traits can describe clonal growth and spreading (Weiher et al. 1999; Kleyer et al. 2008). The lack of focus on clonal traits in FD

studies could be the result of being difficult to measure and not being formed by most trees and annuals (Mudrak et al. 2015; Klimešova and Herben 2015). However, clonal plants are common in temperate, boreal and aquatic vegetation (Klimeš et al. 1997). It has been shown that variation in clonal growth changes between communities (de Bello et al. 2011; Klimešova et al. 2011; 2012), between land use types (Catorci et al. 2011) as well as along large latitudinal, and thus climatic gradients (Ye et al. 2014). Therefore, it is very likely that environmental filters operate on clonal traits (de Bello et al. 2011), as has been shown by Klimešova and Herben (2015) at the community scale.

In this paper our main aim was to study the effect of environmental filtering on LHS vs clonal traits. In particular, we examined patterns of dominant trait values (community-weighted mean, CWM) and functional diversity (FD) in relation to environmental gradients approximated by Ellenberg Indicator Values (EIV). We studied these associations at the habitat type level, because the effect of environmental filtering is assumed to be predominant at such scale and avoids, as much as possible, the potential influence of biotic interactions and hence the effect of limiting similarity.

As both LHS and clonal traits have been demonstrated to respond to environmental conditions, though studied independently from each other, we expected similar response strength for both trait groups. At the same time, LHS and clonal traits should show different responses along particular environmental gradients. A benign environment with sufficient nutrients, light and moisture should provide for a higher diversity regarding above-ground LHS traits, in contrast to more extreme and stressful habitats, where these traits are more strongly filtered. Therefore, we expect FD to be higher in favourable conditions, while in habitats with decreased light, moisture or nutrient availability the FD of LHS traits would be lower. By contrast, clonality as such, as well as a diversity in clonal strategies, could be more beneficial in more stressful and disturbed habitats that are characterized by decreased light and nutrient levels, and increased moisture, in which sexual reproduction might be limited (Herben et al. 2015).

We acknowledge that CWM and FD are likely to be correlated (Ricotta and Moretti 2011; Dias et al. 2013), but we still see value in analysing them independently as no study to date has attempted to quantify their relationship.

Material and methods

Data

Habitats

Species composition in vegetation plots were provided from a standardized and stratified subset (see Herben et al. 2013 for details) of the Czech National Phytosociological Database (Chytry and Rafajova 2003). Plots were assigned to 32 EUNIS habitats according to Chytry et al. (2005). These habitats represent major vegetation types found in Central Europe covering terrestrial as well as aquatic vegetation types. We excluded the three strictly aquatic vegetation types dominated by hydrophytes (surface standing waters, surface running waters and littoral zone) but kept bogs, fens and marshes. Aquatic vegetation types are distinctively different from terrestrial systems, therefore presumed not to behave according to expectations for terrestrial habitats. Moreover, certain functional traits, e.g. plant height, are hard to define in the case of hydrophytes. The selection resulted in 15,711 plots in 29 habitats (Table 1) with occurrence data of 1,812 species. To minimize the effects of interspecific biotic interactions that occur within the plots, we pooled occurrence data at the plot level into frequency data at the habitat level. Finally, the obtained frequencies were transformed into relative frequencies for calculation of CWM and FD indices.

Traits

We obtained two sets of plant functional traits: traits linked to vegetative reproduction (clonal and bud bank traits) and LHS traits. Clonal growth and bud bank traits were extracted from the CLO-PLA database (Klimešova and de Bello 2009, clopla.butbn.cas.cz), whereas LHS traits (specific leaf area, seed mass and plant height) were extracted from the LEDA Traitbase (Kleyer et al. 2008, www.uni-oldenburg.de/en/landeco/research/projects/LEDA). We could retrieve trait data for at least 73% of the species (weighted by their abundance) in the case of all studied traits: SLA 74%, height 75%, seedmass 73%, bud bank traits 95%, and clonal traits 86%. In the following description, trait names and abbreviations used in the remainder of the text are given in brackets.

Table 1 EUNIS habitat types with coding. N plots – number of plots used in the analyses in a certain habitat type. L, T, F, R, N – community-weighted mean of the corresponding Ellenberg values calculated for the habitat types (L – light, T – temperature, F – soil moisture, R – soil reaction, N – soil nutrients).

| Code | Habitat type | N plots | L | T | F | R | N |
|------|--------------------------------|---------|-----|-----|-----|-----|-----|
| D1 | raised bogs | 75 | 7.0 | 4.3 | 8.1 | 2.4 | 2.1 |
| D2 | poor fens and transition mires | 375 | 7.3 | 5.0 | 8.2 | 4.1 | 3.2 |
| D4 | base-rich fens | 49 | 7.3 | 5.1 | 7.7 | 6.2 | 3.4 |
| D6 | brackish marshes | 32 | 7.4 | 5.8 | 8.4 | 7.0 | 6.2 |
| E1 | dry grasslands | 2,414 | 7.5 | 6.1 | 3.4 | 7.1 | 2.8 |
| E2 | mesic grasslands | 1,603 | 7.1 | 5.4 | 5.0 | 5.8 | 4.4 |
| E3 | wet grasslands | 2,207 | 7.0 | 5.2 | 6.9 | 5.4 | 4.3 |
| E4 | alpine grasslands | 50 | 6.3 | 3.8 | 5.5 | 3.1 | 2.6 |
| E5.2 | woodland fringes | 369 | 7.0 | 5.8 | 3.8 | 7.0 | 3.4 |
| E5.4 | wet tall-forb stands | 734 | 6.5 | 5.2 | 7.0 | 6.1 | 5.6 |
| E5.5 | subalpine tall-forb stands | 174 | 5.3 | 3.9 | 5.8 | 5.0 | 5.0 |
| E5.6 | anthropogenic tall-forb stands | 799 | 6.9 | 5.7 | 5.2 | 6.9 | 6.3 |
| E6 | inland saline grassland | 151 | 7.2 | 5.7 | 6.7 | 7.0 | 5.1 |
| F2 | alpine and subalpine scrub | 24 | 5.0 | 4.0 | 5.9 | 4.9 | 4.8 |
| F3 | temperate scrub | 87 | 6.7 | 5.8 | 4.0 | 6.9 | 4.4 |
| F4 | heath lands | 200 | 6.5 | 5.3 | 4.2 | 4.0 | 2.6 |
| F9.1 | riverine willow stands | 20 | 6.3 | 5.4 | 7.1 | 6.6 | 6.8 |
| F9.2 | willow cars | 48 | 6.6 | 5.2 | 7.9 | 5.5 | 5.2 |
| G1 | broad leaved woodland | 1,615 | 4.7 | 5.4 | 5.3 | 6.0 | 5.2 |
| G1.C | broad leaved plantations | 27 | 5.2 | 5.5 | 5.6 | 6.4 | 6.2 |
| G3 | coniferous woodland | 385 | 5.1 | 4.6 | 5.4 | 3.9 | 3.7 |
| G3.F | coniferous plantations | 163 | 5.3 | 5.4 | 4.8 | 5.5 | 4.5 |
| G4 | mixed woodland | 856 | 4.6 | 5.2 | 5.3 | 5.6 | 5.1 |
| G5 | forest clearings | 448 | 5.7 | 5.4 | 5.4 | 5.1 | 5.4 |
| H2 | screes | 50 | 7.1 | 5.8 | 3.7 | 6.8 | 3.7 |
| H3 | cliff and outcrops | 236 | 6.5 | 5.5 | 4.4 | 6.1 | 4.0 |
| H5.6 | trampled areas | 733 | 7.3 | 5.8 | 5.8 | 6.2 | 5.7 |
| I1 | arable land | 1,397 | 6.7 | 5.8 | 4.9 | 6.6 | 6.1 |
| J6 | waste deposits | 390 | 7.4 | 6.0 | 4.8 | 6.9 | 6.4 |

Traits of the leaf-height-seed (LHS) strategy scheme

Specific leaf area (SLA), defined as the leaf area per dry weight, is positively correlated with the potential growth rate and therefore considered to represent different resource acquisition strategies (Westoby et al. 2002).

Canopy height (Height) describes the mean distance between foliage of a plant and the soil surface. In competitive environments, higher growing plants have an advantage through prior access to light (Westoby et al. 2002). Seed mass is the dry mass without accessories. Heavy seeds have high metabolic reserves which favours the establishment of seeds in productive and competitive environments (Willson and Traveset 2000).

Bud bank traits

Root sprouting capacity (Root spr.) is a binary trait, coding the ability of a plant to resprout from root-derived buds after the destruction of its aboveground biomass. Bud bank size (Sum BB) is defined as the number of vegetative buds per shoot, with these buds residing on belowground stem-derived organs. Bud bank size including buds on roots (Sum BBR) adds the Sum BB and root-derived buds of a plant. Bud bank depth (Depth BB) and bud bank depth including root buds (Depth BBR) describe the depth of buds in relation to the soil surface. The crucial role of the bud bank in regeneration after a disturbance event has been studied in different habitats. Bud banks can increase a plant's distribution by allowing new shoots to be established away from the mother plant; in addition, bud-bearing organs contain carbon reserves used for seasonal regrowth, respiration, flowering and fruiting. Thus, regrowth from reserve meristems is also a successful strategy in severely and repeatedly disturbed habitats (Klimešová and Klimeš 2007; Klimešová and de Bello 2009).

Clonal traits

Multiplication rate (Mult. Rate) is defined as the number of offspring shoots per parent shoot per year (Klimešová and de Bello 2009) and lateral spread (Lat. Spread) is the distance a clonal organism spreads each year. Together, these two traits are combined to give the clonal index (Clo. Index), which is an overall measure of how clonal a plant behaves. For its calculation, lateral spread is assigned to one of four ordinal classes (less than 1 cm, 1–25 cm, more than 25 cm, freely dispersible), multiplication rate to three (less than one offspring, one offspring, more than one offspring), and the clonal index is a summation of the two resulting values for a species. Although clonal growth is achieved through various morphological adaptations and strategies, these three

measures of clonality express its main components in terms of function: vegetative reproduction, the exploitation of space and resources, and local persistence (van Groenendael et al. 1996).

Trait combinations

LHS traits (LHS) combine the traits SLA, height and seed mass. Bud bank traits (BB) include the traits root spr., sum BB, sum BBR, depth BB and depth BBR. Clonal traits (Clonal) include the traits Mult. Rate, Lat. Spread and Clo. Index. Clonal and bud bank traits together (Clo. + BB) combine all clonal and bud bank traits.

Community-weighted mean and functional diversity

Traits of the LHS strategy scheme were log-transformed as their values ranged between several orders of magnitude and they had a lognormal distribution. All numeric traits of the clonal and bud bank traits were standardized to zero mean and unit variance. Species that were known to be not clonal received a value of zero for the clonal traits multiplication rate, lateral spread and the clonal index. We used Rao’s quadratic entropy (Rao, Botta-Dukát 2005) as index of FD. Rao is an index of functional dissimilarity,

expressed as the sum of the abundance-weighted pairwise differences between species of a community

$$Rao = \sum_{i=1}^n \sum_{j=1}^n d_{ij} \rho_i \rho_j$$

where d_{ij} is the functional distance between species i and j and p_i and p_j are the relative abundances of the i -th and j -th species. The Rao index was calculated for both individual traits as well as for three combinations of ecologically related traits: LHS traits, bud bank traits, clonal traits and the combination of clonal and bud bank traits (see also Table 2). We calculated the community-weighted mean (CWM, Garnier et al. 2004) for the transformed traits, weighted by the relative abundance of species in habitats

$$CWM = \sum_{i=1}^n \rho_i x_i$$

where x_i is the mean trait value and p_i is the relative frequency of the i -th species. The CWM for the binary trait root sprouting ranges between 0 and 1, representing the abundance-weighted fraction of species carrying the value 1 (i.e. being capable of root sprouting).

To characterize average environmental conditions in the 29 habitat types, we used the CWM of Ellenberg

Table 2 Expected (Exp.) and observed (Obs.) R^2 values for linear models on community-weighted mean (CWM) and functional diversity (Rao) with linear and quadratic EIV

| | CWM | | Rao | |
|------------------------------------|------------|-------------|------------|-------------|
| | Exp. R^2 | Obs. R^2 | Exp. R^2 | Obs. R^2 |
| Specific leaf area (SLA) | 0.33 | 0.88 | 0.32 | 0.41 |
| Height | 0.33 | 0.14 | 0.33 | 0.39 |
| Seed mass | 0.33 | 0.40 | 0.33 | 0.50 |
| LHS traits | NA | NA | 0.33 | 0.29 |
| Root sprouting capacity | 0.34 | 0.86 | 0.35 | 0.88 |
| Bud bank size | 0.33 | 0.46 | 0.35 | 0.75 |
| Bud bank size including root buds | 0.34 | 0.38 | 0.35 | 0.81 |
| Bud bank depth | 0.31 | 0.29 | 0.32 | 0.80 |
| Bud bank depth including root buds | 0.34 | 0.17 | 0.34 | 0.88 |
| All bud bank traits | NA | NA | 0.33 | 0.81 |
| Multiplication rate | 0.33 | 0.76 | 0.34 | 0.47 |
| Lateral spread | 0.35 | 0.62 | 0.32 | 0.84 |
| Clonal index | 0.33 | 0.88 | 0.37 | 0.84 |
| All clonal traits | NA | NA | 0.32 | 0.78 |
| Clonal + bud bank traits | NA | NA | 0.34 | 0.79 |

Significantly better Obs. than Exp. R^2 are shown in bold ($SR^2 > 1.65$, $P < 0.05$).

Indicator Values (EIV, Ellenberg et al. 1992; Table 1), calculated the same way as for the traits. EIV are empirical ordinal values of plant species representing their preferences along environmental gradients. We used here EIV of light availability (L), soil moisture (F), soil reaction (R), temperature (T) and soil fertility (N). Each EIV ranges from one (lowest value) to nine (highest value). For example, L1 species occur primarily in shaded habitats, while L9 species are found in habitats with extremely high light availability. Small R values indicate more acidic soils (small pH values), while higher R refers to more basal soils (higher pH values). The EIV for moisture extends the range from one to twelve, with the three highest values indicating the habitat preferences of aquatic plants, which were, however, excluded from this study. In the remainder of the text we use short names or abbreviations for EIVs given above. It is important to note that these gradients reflect species preferences and not direct measures of any environmental variable. Thus, the CWM of L for a habitat type refers to the weighted average preference of species in that habitat not the actual level of light available.

Data analysis

Models

While the literature provides guidance for hypotheses regarding trait-environment relationships, this is less well developed for clonal traits. As a result, in this analysis we took an exploratory approach and thus, to relate CWM and FD to environmental properties (expressed as EIV), we used linear regression with stepwise forward selection to find the most parsimonious model. To counteract the drawbacks of stepwise model selection (see e.g. Mundry and Nunn 2009), stepwise selection was based on Akaike information criterion (Burnham and Anderson 2002), and we reduced the number of our predictors as much as reasonable (see next paragraph).

Initially, using single EIV and their quadratic term as explanatory variables, we tested for the presence of unimodal relationships. Scatterplots of these models, if significant, are depicted in the [Electronic Supplementary Material](#). We found only three non-linear relationships (i.e. only the model including the quadratic terms was significant) of all 100 tested combinations, thus we decided to omit these from further analyses. This exclusion allowed for more reliable results, as stepwise models with high

numbers of predictors tend to select irrelevant variables for the minimum adequate model. To further improve the interpretability of predictor variables in terms of their relative importance, they were centred and standardized (Schielzeth 2010). In the following, we refer to the R^2 of these models as the observed R^2 (see also next section).

To analyse the relationship between FD and CWM we used simple linear models, with FD as the response variable and CWM as the predictor, where CWM was entered as a linear and as a quadratic term, as the expected relationship between FD and CWM is unimodal, but can also be linear if the composition of the communities does not include all possible combinations of species (see Dias et al. 2013).

Randomizations

As relating EIV to other metrics derived from species composition can produce confounded results (Zelený and Schaffers 2012), we compared the observed R^2 of final models to the R^2 expected by chance. To derive expected R^2 , the stepwise selection method was performed on the same variables, but CWM and FD of traits have been calculated from randomized trait values. Randomization was achieved by shuffling species identities in the species by trait table, thus keeping trait combinations intact, but assigning sets of trait values randomly across all species. Species richness and abundance structure of the habitats are maintained by this procedure. The indices were related to the EIV using the same stepwise regression procedure as on observed species occurrence data, keeping the original centred EIV of habitats. For the R^2 of models on habitats, the mean (referred to as expected R^2) and standard deviation of R^2 were calculated from the distribution of R^2 values from 999 randomizations.

To judge whether the observed models are significantly better than randomized, a standardized R^2 (SR^2) was defined as

$$SR^2 = \frac{(\text{Observed } R^2 - \text{Mean Expected } R^2)}{SD \text{ Expected } R^2}$$

Observed R^2 were considered significantly larger than expected if $SR^2 \geq 1.65$ (based on one-sided t-test with $\alpha = 0.05$).

RDA

Redundancy Analysis (RDA) was used to display the relations between environmental gradients and CWM and FD. For each response group, defined as CWM and Rao of traits in habitats, we performed a single analysis. The standardized response variables were constrained by habitat CWM of EIV light availability (L), temperature (T), soil moisture (F), soil reaction (R) and soil nutrients (N). All analyses except RDA were done in R v. 3.2.2 (R Core Team 2015) and package ‘FD’ (Laliberté et al. 2014), the RDA’s were done with CANOCO v. 5.04 (Microcomputer Power, Ithaca, NY, US).

Results

Comparison of the observed R^2 and the expected R^2 showed that models predicted the observed CWM better than expected by chance in only four out of eleven cases (Table 2). We found at least one trait significantly related to environmental gradients in each group of LHS, bud bank, and clonal traits (SLA, root sprouting capacity, multiplication rate and clonal index, respectively). By contrast, the models on Rao had significantly higher R^2 than expected by chance for almost all clonal and bud

bank traits (the only exception being multiplication rate), but for none of the LHS traits.

There was no significant relationship between CWM and FD for any of the LHS traits, but for all clonal and bud bank traits, except multiplication rate. For root sprouting capacity, bud bank size, bud bank size including root buds, and bud bank depth including root buds the relationship was curvilinear, whereas for bud bank depth, lateral spread and clonal index there was a linear relationship of CWM and FD (Fig. S1 in the Electronic Supplementary Material).

The performed RDAs display the links between environmental gradients (expressed as EIV) and CWM and Rao. In both analyses, a substantial amount of variation was explained by the first two axes, with 56.5% in case of CWM and 76.9% in case of Rao, respectively.

The first axis in the RDA on CWM (Fig. 1) is strongly related to soil nutrient content (N). SLA and multiplication rate are also positively correlated with N. The second axis is mainly related to soil moisture (F). Increasing values of F are accompanied by a higher proportion of root sprouting plants, and a higher multiplication rate and clonal index.

Much of the variation in Rao of traits is explained by the first axis (71.1%), with the second axis contributing by only a small amount to the variation explained by the RDA (Fig. 2). The EIV for

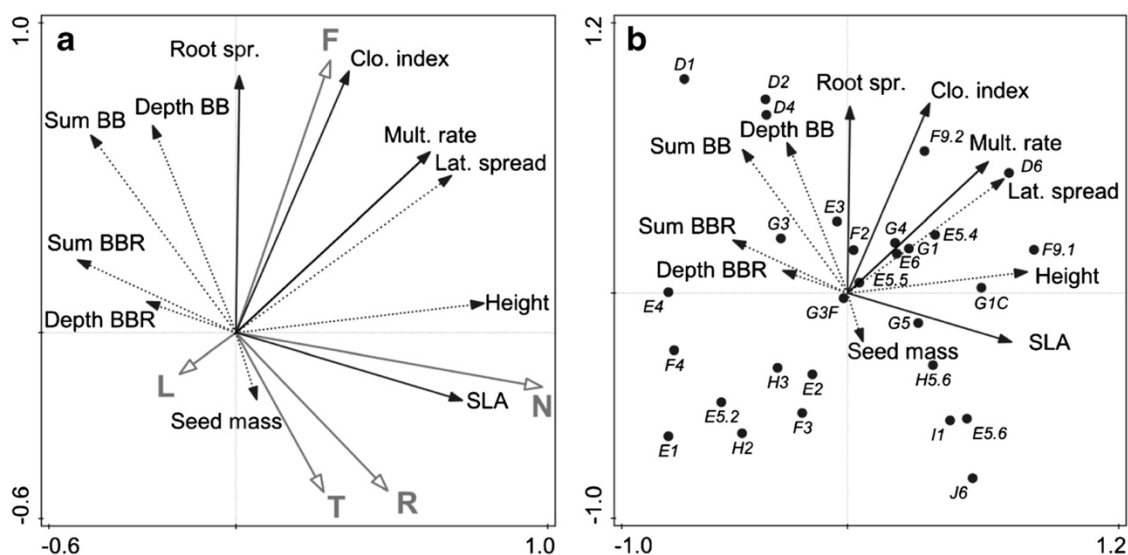


Fig. 1 RDA on CWM for traits using EIV L, T, F, R and N as constraining variables. The first axis explains 32.7%; both axes together explain 56.5% of variance in trait CWM of habitats. When the trait-environment relationship was not different from

one expected by chance (i.e. non-significant SR^2), the arrow is dotted. Besides traits, graph **a** displays constraining EIV, and graph **b** displays the 29 habitats. For habitat coding, see Table 1.

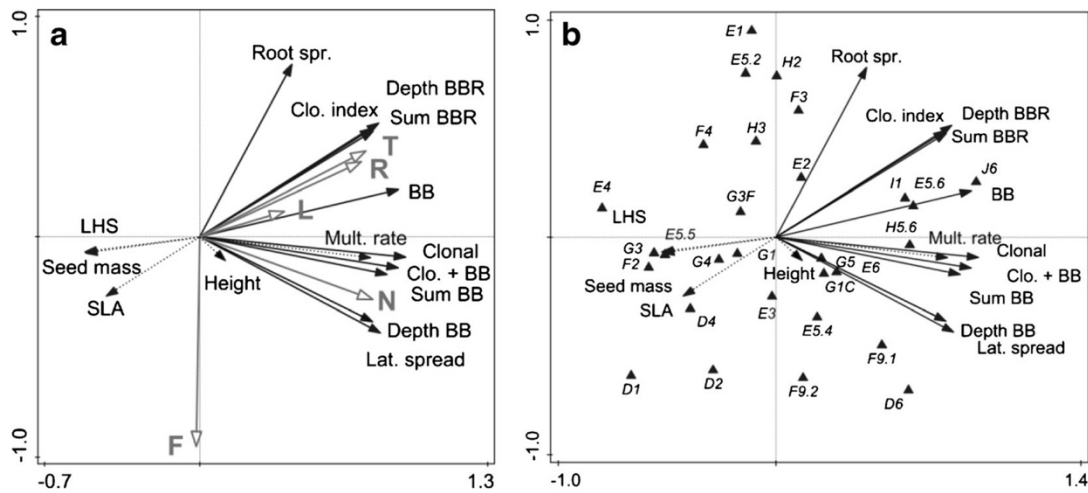


Fig. 2 RDA on Rao for each individual trait and trait combinations using EIV L, T, F, R and N as constraining variables. The first axis explains 71.2%, both axes together explain 76.3% of variance in trait CWM of habitats. For traits that had a non-significant SR^2 ,

the arrow is dotted. Besides traits, graph **a** displays constraining EIV. Graph **b** displays the 29 habitats. For habitat coding, see Table 1.

temperature (T), soil reaction (R), soil nutrients (N) and less pronounced light (L) are positively correlated to the first axis. Rao of clonal and bud bank traits and their trait combinations all (except for multiplication rate) increase in the same direction. Similarly to the RDA on CWM, the second axis

mainly represents a moisture gradient (F), to which the Rao of root sprouting is negatively correlated.

In most models that included clonal and bud bank traits' CWM and Rao, the EIV for temperature, moisture and nutrient availability were highly significant predictors (Table 3).

Table 3 Summary of linear models between EIV and CWM and Rao. Cells contain slope estimates for each EIV retained in the most parsimonious model after forward stepwise selection. NA stands for 'not available', as CWM are not meaningful for multiple

traits. Highlighted in bold font are the models for which observed R^2 did deviate from expected R^2 (see Table 2). Asterisks indicate the significance of the linear terms. * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$.

| Index | CWM | | | | | Rao | | | | |
|------------------------------------|-----------------|----------------|----------------|---------------|----------------|----------------|----------------|-----------------|---------------|----------------|
| | L | T | F | R | N | L | T | F | R | N |
| Specific leaf area | -0.37*** | | | 0.15** | 0.29*** | -0.26* | 0.25 | 0.27** | | -0.34** |
| Height | | | 0.11 | | 0.39*** | | | | | |
| Seed mass | -0.26*** | | -0.12** | | | -0.19** | 0.15 | | | -0.28** |
| LHS traits | NA | NA | NA | NA | NA | | | | | -0.44** |
| Root sprouting capacity | | 0.17*** | -0.2*** | | | | 0.3*** | -0.44*** | | 0.11 |
| Bud bank size | -0.34** | | 0.34*** | 0.14 | -0.54*** | 0.14* | 0.23* | | -0.13 | 0.36*** |
| Bud bank size including root taps | -0.14* | | | | -0.25** | 0.15* | 0.31** | -0.27*** | -0.12 | 0.34*** |
| Bud bank depth | -0.26** | | 0.26*** | 0.13 | -0.32** | 0.11* | 0.21** | 0.11* | -0.1 | 0.24*** |
| Bud bank depth including root taps | -0.14* | | | 0.11 | -0.18* | 0.15*** | 0.22*** | -0.23*** | -0.11* | 0.28*** |
| All bud bank traits | NA | NA | NA | NA | NA | 0.59** | 0.88** | -0.39* | -0.37 | 1.1*** |
| Multiplication rate | -0.06 | 0.14* | 0.29*** | | 0.08 | 0.09** | | | | 0.2*** |
| Lateral spread | | | 0.3*** | | 0.25*** | | 0.47*** | 0.27*** | -0.16 | 0.51*** |
| Clonal index | -0.15*** | | 0.35*** | | | 0.31*** | 0.13 | -0.3*** | -0.16* | 0.36*** |
| All clonal traits | NA | NA | NA | NA | NA | 0.44** | 0.67** | | -0.38* | 1.1*** |
| Clonal and bud bank traits | NA | NA | NA | NA | NA | 0.71** | 0.91** | | -0.64* | 1.55*** |

Discussion

LHS and clonal traits in relation to environmental gradients and habitat types

In this paper we explored the relationship between environmental gradients and LHS and clonal traits across 29 temperate habitat types. We investigated associations for both the dominant trait values (CWM) and functional diversity characterizing those habitat types. The prevailing environmental gradients across habitats were related to soil productivity and moisture. This is consistent with findings of Klimešová and Herben (2015), who used the same data source but analysed trait-environment relationships at the plot level and did not use EIV. Our results suggest that most clonal and bud bank traits are significantly related to all studied environmental gradients. Moreover, these environmental gradients are mostly associated with the diversity of clonal and bud bank traits, but less with the dominant trait values or categories.

By contrast, out of the three LHS traits only CWM of SLA was significantly associated with a single EIV (soil nutrients). These differing results imply that the variation of traits related to vegetative reproduction responds stronger to environmental conditions than the studied aboveground traits.

Use of Ellenberg indicator values

In this study, Ellenberg indicator values were used to characterize environmental conditions of habitats. EIV has been shown to be a useful proxy for environmental variables in several previous studies (see e.g. Schaffers and Sýkora 2000; Tölgyesi et al. 2014). However, EIV are subject to a long debate over whether they are suitable for describing environmental conditions, being derived from empirical experiences about species preferences and distribution patterns rather than based on real measurements (Zelený and Schaffers 2012; Wildi 2016). Exner et al. (2002) discussed that using EIV for habitat characterization based on community composition involves circularity, as species occurrences are involved to generate both, response (community composition) and explanatory (mean EIV) variables. Therefore, we used randomizations to overcome the problem of spurious results. For the discussion,

we refer to EIV gradients (obtained by calculating CWM of each indicator value for every habitat type) as environmental gradients: L being light availability, T temperature, F moisture, R soil reaction and N being nutrient availability or productivity of the habitat.

Community-weighted means

Regarding the predominant trait values in different habitats, we found two main gradients in our study, at one end indicating cold and wet conditions (such as bogs, fens and woodlands) while at the other end disturbed, more productive and thermophilous habitats (such as arable lands and trampled areas). Our finding that productive habitats hosted species with higher SLA values is in line with previous studies (e.g. Wilson et al. 1999) indicating higher resource acquisition and a less conservative growth strategy in these habitats (Pérez-Harguindeguy et al. 2013). By contrast, less productive habitats (high F, low T and R) such as bogs and fens, were dominated by species with lower SLA values, indicating that the most abundant species in these more stressful habitats show a more conservative strategy.

Species with clonal growth were more frequent in wet and cold habitats, in accordance with the results of van Groenendael et al. (1996) and Ye et al. (2014). Our results showed increased root sprouting, multiplication rate and clonality, which in general might serve as an alternative to sexual reproduction for several reasons. For instance, in habitats under harsh environmental conditions, as alpine/subalpine and arctic scrublands, clonal growth can ensure reproductive success. At the same time, such habitats expose clonal plants to low levels of disturbance, which favours their establishment and persistence (Callaghan et al., 1992; Klimešová et al. 2011; Klimešová et al. 2012). In woodlands of the temperate regions the time window for pollination and sexual reproduction is often short and limited to early-year unshaded conditions (e.g. Catorci et al. 2012). Also, the recruitment of species without persistent seed banks is supported mostly by clonal organs, allowing establishment in the spatially unpredictable patches of forests (Eriksson 1989).

Considering grasslands among the studied habitats, the clonal index increased from xeric through mesic to wet grasslands, which corroborates a study by Halassy

et al. (2005) who found higher proportions of species with short spacers in xeric compared to mesic grasslands. By contrast, Wellstein and Kuss (2011) as well as Klimešová and Herben (2015) found more species with short spreading in relatively wetter, but also considerably colder, high-elevational alpine habitats, which are not represented in our dataset.

Multiplication rate was also positively correlated with productivity. Among others, Klimešová et al. (2011) and Craine et al. (2001) proposed that more fertile habitats favour species with higher competitive ability both above and below ground, leading to species with a higher number of clonal offspring.

Functional diversity

Clonal traits were significantly more diverse in environments that represented high temperature, soil reaction and fertility (Fig. 2). Despite the tendency for species to be less clonal (see CWM section) in habitats with these conditions (trampled areas, arable lands, waste deposits and anthropogenic tall-forb stands), the species that do form clonal organs and bud banks seem to use different strategies to cope with the unpredictability of occurring disturbances in these habitats.

In rather undisturbed habitats (alpine and subalpine environments, bogs and fens), conditions of low temperature, soil reaction and fertility act as filters that reduce the diversity in clonal traits. In these habitats pollinator availability might be limited, either because of the low temperature at high altitudes, or due to larger open water surfaces (in the case of fens, bogs, wetlands). Even in habitats where pollinators are available, low productivity might lead to a lack of flowering species (Fujita et al. 2014). Therefore, lateral spread might offer an alternative (vegetative) way of reproduction when sexual reproduction is limited (Herben et al. 2015).

Soil moisture constitutes another gradient associated to the diversity of clonal strategy. In moister habitats, species have more diverse lateral spread strategies compared to xeric and rocky habitats (dry grasslands, woodland fringes, cliffs and outcrops). In the latter habitat types, a patchy distribution of nutrients might constitute a filter that can be passed more likely by species with the ability to access nutrients by clonal spreading, a strategy that has been proposed for several systems (reviewed in Liu et al. 2016).

LHS traits were less diverse across the gradients than clonal traits. Moreover, relationships of LHS traits with

environmental gradients were not significantly different from patterns expected by chance. In temperate Europe, the three LHS traits show a relatively small range, whereas the divergence in clonal and bud bank traits could allow co-existence of species that are similar above ground but dissimilar below ground e.g. in depth of bud bank, and in shape and size of rhizomes (Klimešová et al. 2011). This adds evidence to the recently expressed view that clonal and bud bank traits could represent an important independent axis of plant strategy (Klimešová et al. 2016).

Theoretically, such a finding could be the result of clonal and bud bank traits usually being recorded more discretely, i.e. with a lower number of unique values along the continuous trait scale, compared to LHS traits. In our dataset, single clonal and bud bank traits are indeed less continuous than LHS traits, leading to a more right-skewed distribution of distances calculated from LHS traits compared to clonal and bud bank traits. While this might in fact contribute to a stronger expression of variation in clonal and bud bank traits, it should not translate into an inability to detect filtering for LHS traits if it would exist, as FD of traits and its association to environmental gradients was investigated in separate models for each trait.

Relationship of CWM and FD for LHS, clonal, and bud bank traits

The finding that clonal and bud bank traits respond more strongly to environmental gradients is also reflected by significant relationships between CWM and FD for these traits. While it has to be noticed that this relationship is based on a mathematical relationship between the two metrics, it is nevertheless possible to refer to this relationship to indicate if a change in one of the metrics is biologically connected to a change in the other.

The whole range of the hypothetical unimodal relationship has been demonstrated by simulated communities (see Dias et al. 2013), whereas the empirical patterns found here all seem to fall on subparts of the entire possible curve. This at least enables us to qualify if observed significant changes in CWM are associated with FD. Thus, there are three different scenarios for the relationship of the two metrics: (i) a change in FD but not in CWM, i.e. there is filtering on trait variation but not on the allowed trait values; (ii) changes in both FD and CWM, i.e. filtering acts on the allowed traits and their variation; (iii) a change only in CWM but not in

FD, indicating that habitat conditions filter out specific trait values, but has no effect on how different these traits are within a habitat.

Our finding that except for SLA, LHS traits are not filtered in the studied habitats are mirrored by the fact that their CWM and FD are unrelated to each other. By contrast, the capacity for root sprouting, and a higher clonal index, bud bank size and bud bank depth coincide with a decreased FD in these traits. This shows that even though CWM for some of these traits was only spuriously correlated with environmental gradients, it is not always straightforward to tease apart if traits respond to gradients by shifts in the trait values of the dominant species or by a reduced variation among the species.

Conclusions

Our analyses revealed more consistent and significant relationships between clonal traits and environmental gradients than between LHS traits and environmental gradients. We therefore propose that there is a stronger filtering on clonal than on LHS traits, as the former tend to be less diverse within habitats that have decreased temperature, pH, nutrient levels and light. These unfertile, harsher environments are all favoured by plant species that are able to spread vegetatively. At the opposite end of these environmental gradients, clonal and bud bank traits are more diverse indicating that the filtering is weaker and more clonal strategies exist in parallel in more fertile habitats.

As such, our results hint at clonal and bud bank traits being an understudied dimension of plant strategy, which are not included in classical strategy schemes as C-S-R (distinguishing competitive, stress-tolerant and ruderal; Grime 2006) or the LHS scheme (Westoby 1998). We propose that clonal and bud bank traits could indeed be added as an independent axis to functional trait space capturing plant form and function (Laughlin 2014).

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Species composition vs functional trait variation along temporal gradient: permanent plot-based approach

Bricca¹, A., Cutini¹, M.,

Affiliation

¹Department of Science, University of Roma Tre, Rome, Italy

ale.bricca@gmail.com (<https://orcid.org/0000-0003-0202-6776>)

maurizio.cutini@uniroma3.it (<https://orcid.org/0000-0002-8597-8221>);

INTRODUCTION

Predicting ecosystem responses to global change, such as land use and climate, is a major challenge in ecology (Lavorel et al., 2002; Suding et al., 2008) and critical step in that challenge is to understand how changing environmental conditions influence processes of vegetation dynamics. Studies on vegetation dynamics are generally based on “indirect” observation, i.e. on the comparison of sites along land-use gradients or climatic gradients (space-for-time substitution or chronosequences; Laliberté, 2010; Catorci et al., 2011; Sternberg et al., 2011). This approach typically involves extrapolation from spatially distinct sites that are expected to represent certain stages in a temporal succession (Pickett, 1989). On the contrary, the “direct” observation involves monitoring of dynamic, long-term vegetation changes (Schmidt, 1988), which necessarily involves long study periods. However, patterns of vegetation changing over time are not sufficiently understood because most studies are based on space-for-time substitution (Wesche et al., 2012). Indeed, existing environments as proxies for environments under future changed are not, in themselves, sufficient to predict changes in vegetation (Sternberg et al., 2011). Contrary, long-term studies on natural gradients are considered to be powerful tools for monitoring changes in biodiversity, exploring the causes beyond those changes and assessing the conservation status of particular habitats (Giarrizzo et al., 2016; Sperandii et al., 2017). Different approaches have been developed to investigate long-term changes in the vegetation (Chytrý et al., 2014), and very few studies with the permanent plots (Naaf & Wulf, 2011; Wesche et al., 2012).

Furthermore, long-term studies are mainly based on species diversity approach (Britton et al., 2009; Pauli et al., 2012; Sperandii et al., 2017). Generally, species richness is considered as only measure of biodiversity (Balvanera et al., 2006), asserting a positive relationship with ecosystem functioning and services (Diaz & Cabido, 2001). However, evidences about species loss affecting ecosystem functioning and services via species traits are increasing (Chapin et al., 2000; Hooper et al., 2005; Suding et al., 2008; de Bello et al., 2010; Laliberté

et al., 2010). Therefore, assessing ecosystem functioning with a species composition approach may lead to misleading interpretation. Indeed, coexistence species may show similar traits (“functional redundancy”; de Bello et al., 2009), therefore functional traits variation could not be inferred from species diversity variation (de Bello et al., 2009; Carmona et al., 2012; Malavasi et al., 2016). Thus, the decreasing of species with similar strategy probably will not affect the ecosystem functioning and services (de Bello et al., 2009; Laliberté et al., 2010). While the impact of global change has been mostly quantified on species loss (Pimm & Raven 2000; Pauli et al., 2012; Evangelista et al., 2016; Sperandii et al., 2017), its impacts on functional traits over time remain much less clear as well as on the link between species diversity and functional traits. Indeed, to date few studies have attempted to quantify temporal shift in community functional traits (Naaf & Wulf, 2011; Giarrizzo et al., 2016).

Globally, changing land-use patterns is the most important driver of biodiversity loss (Sala et al., 2000). For long time, anthropogenic actions such as deforestation, burning, grazing and mowing created and maintained European grassland (Dengler et al., 2014) and their ecosystem services, such as fodder provision, soil stability and fertility, C sequestration, as well as water and climate regulation (de Bello et al., 2010a). Currently throughout Europe, semi-natural grassland are threatened by the cessation of traditional land use and land abandonment (Sebastià et al., 2008), affecting the current structure of the landscapes with reforestation processes (Brachetti et al., 2012; Malavasi et al., 2018) and promoting the invasion of coarse tall grasses species at finer spatial scale (Catorci et al., 2011). This process is relevant on Mediterranean mountains, where many of the species and plant communities are of concern for the conservation of natural biodiversity in Europe (e.g., protected by the European Commission (1992) network and European protected areas) (Tsiafouli et al., 2013). The degradation is being occurred at very high rate and land abandonment has become a major issue in biodiversity conservation throughout Europe (e.g. Catorci et al., 2011; Vitasović Kosić et al., 2011; Peco et al., 2012).

This research sets out to describe temporal shift in community functional traits and species diversity which occurred over the last decade in Mediterranean mountain semi-natural grasslands. We applied a direct approach, selecting permanent plots along elevation gradient on Velino massif (Central Italy), since this massif is representative of the vegetation dynamics occurring in the Mediterranean mountains in the last centuries (Malavasi et al., 2018). Our aims were to: (1) analyse changes in species composition and species functional traits occurred during the last decade; (2) identify the main directions of these changes.

METHODS

Study Area

The study area is Mt. Velino Massif (2486 m a.s.l.), located in the Central Apennine (Abruzzo region; Fig.1), Italy. The limestone massif is characterized by a sub-Mediterranean climate defined by a summer drought period and cold-stress during the winters occurred with different intensity and timespan along the elevation

gradient (Petriccione, 1993; Theurillat et al., 2011; Table 1). The Velino massif is part of the Sirente-Velino Natural Regional Park (54391 ha), instituted in the 1989, and the study area is included within the Natura 2000 national network (IT110206 “*Monte Sirente e Monte Velino*”). The upper part of Monte Velino, in particular on the south-west slope, is also an Italian site of the E-LTER (European Long Term Ecological Research) monitoring network (Rogora et al., 2018). Since 2005 a monitoring project (“Elevation gradient of vascular plants distribution in the Central Apennines: diversity patterns and potential impact of global change”) along the south-west slope, supported by Italian Ministry of Education, Universities and Research (see also Theurillat et al., 2011). Therefore, the area represents a worthwhile training ground to assess temporal changes in mountain ecosystems

The area is almost deforested above 1200 m asl. It has been subject since millennia to grazing activities that peaked in the mid-19th century, declined strongly in the 1950s due to the abandonment of transhumance practices (Petriccione, 1993), with a further decline on pastoralism during the last decades, following socio-economic transformations due to the decline of the human population in the surrounding region. The vegetation is represented by secondary dry grasslands with *Sesleria juncifolia*, *Globularia meridionalis*, *Carex humilis*, *Bromus erectus*, dominated from the lowest part of the area, up to ca. 2000 m a.s.l. Above 2000 m a.s.l., small elevation section is dominated by primary grassland with cryophilous species (e.g., *Carex kitaibeliana*) and alpine elements such as *Silene acaulis* and *Potentilla crantzii* (Petriccione, 1993).

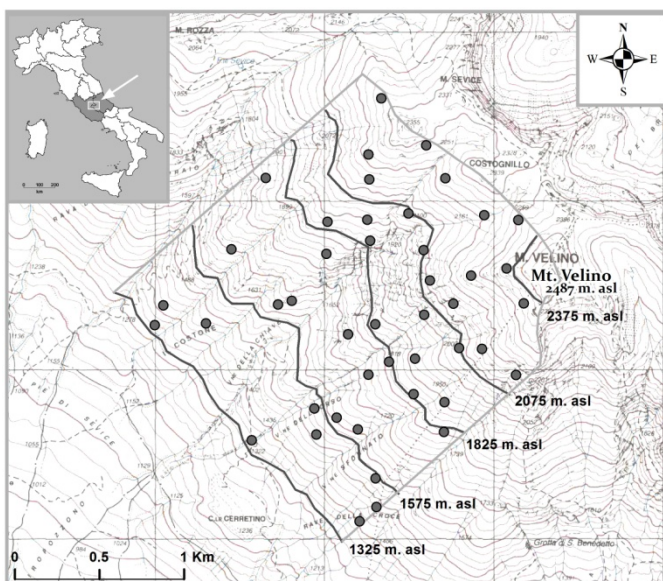


Fig.1. The rectangle represents the study area (Velino Massif, Central Italy) and continuous line represent the 100 m elevation bands of the MIUR Project. Dots are the subset of 45 permanent plots of the MIUR Project selected for the sampling.

| Termopluviometric station | 1 | 2 | 3 | 4 |
|---------------------------|--------|--------|--------|--------|
| Altitude (m a.s.l.) | 1450 | 1700 | 1950 | 2200 |
| T (°C) | 15.02 | 12.92 | 11.47 | 7.79 |
| T winter (°C) | 2.17 | 0.06 | -0.40 | -3.46 |
| MAT | 8.64 | 6.52 | 5.50 | 2.06 |
| P (mm) | 434.28 | 419.07 | 456.36 | 455.64 |
| PET (mm) | 89.53 | 85.40 | 78.86 | 72.17 |
| Aridity Index (P/PET) | 1.07 | 1.09 | 1.31 | 1.53 |

Table 1. Climatic characterization of the study area. Mean values of air temperature (T), sum annual precipitations (P), potential evapotranspiration (PET), aridity index during the growing season (May to September). T winter = mean values of air temperature during the winter season from November to February; MAT = mean annual temperature.

Vegetation and functional traits sampling

Floristic sampling events took place every five years: 2006, 2011 and 2016. During 2016 we selected a subset of permanent plots initially established in 2006, to exclude inter-plot environmental heterogeneity along the elevational gradient, according to the following criteria: open calcareous grassland, absence of domestic grazing, south-west aspect (227 ± 43 degrees), almost the same slope values (33 ± 4 degrees). This resulted in 45 2x2 m plots positioned from 1,325 m a.s.l. to 2,375 m a.s.l. From May to August 2016 we conducted a floristic survey on these 45 plots (2x2 m), collecting species presence data and visually estimating species relative species cover. The same scale used in previous floristic sampling for estimating species cover was used also in the 2016. Prior to analysis, these records were transformed to percentage values as follow: +: 0.1%, **1a**: 2.5%, **1b**: 5% **2a**: 10%, **2b**: 20%, **3a**: 31.25%, **3b**: 43.75% **4a**: 56.25%, **4b**: 68.75%, **5a**: 81.25%; **5b**: 93.75%. As measuring traits for all species in each plot was infeasible, we divided the elevation gradient into four sections of almost 250 m. The first section (1325-1575 m a.s.l.) had 9 plots, 10 plots were positioned in both the second (1575-1825 m a.s.l.) and 11 third (1825-2075 m a.s.l.) sections, while 15 plots were located the last section (2075-2375 m a.s.l.) (Fig.1). Then, we followed the framework of Pakeman & Quested, (2007) but at belt scale, pooling together all the plots belonging to the same belt and selecting for traits measurement those species whose cumulative cover reached 80% of the total vegetation cover of the belt.

We measured traits related to the performances of competitive ability (plant height) and rate of resource investment (specific leaf area) for a total of 50 species. In detail, we measured plant height (cm) in 15 individuals, SLA (mm^2/mg) in 10 healthy, fully expanded leaves in each of 10 different individuals. All the individuals were gathered in the middle quote of each elevation section, respecting the same topographic features (slope, aspect, land form) of the plots, and were measured according to international standardized trait measuring protocol (Perez-Hargundegay et al., 2013).

We followed this same approach (i.e. same elevation section, same plot identity, same number of plot for belt) for the previously two events of floristic sampling occurred in 2006 and 2011. We retrieved floristic data from

Laboratory of Vegetation Ecology of RomaTre. The total number of measured species for the 2006 was 37 and for 2011 was 46.

All the species has been measured during the growing season of 2016 according to international standardized trait measuring protocol (Perez-Hargundegay et al., 2013) and according to the abovementioned number of individuals per species. We were not able to consider also the intraspecific trait variability, thus the variation in community functional traits reflect only variation in species composition (cover and taxonomic identity).

Statistical analysis

The functional composition of a community is commonly investigated with a measure of functional composition, i.e. community-weighted mean (CWM; Garnier et al., 2004) and a measure of functional diversity, i.e. functional dispersion (FDis; Laliberté & Legendre, 2010). We computed the indices for each permanent plot using species cover at plot scale and trait values collected at the mid-point of each elevation belt for each of the floristic sampling period, *i.e.* 2006, 2011 and 2016. The community-weighted means for each trait (hereafter CWM_H, CWM_{SLA}) were calculated with the following equation of Garnier et al., (2004):

$$CWM = \sum_{i=1}^n p_i x_i$$

where *CWM* is the community-weighted trait value of a given functional trait, p_i is the relative abundance in the plot of the i -th species and x_i is the mean trait value of the species i . This index reflects the trait-environmental relationship quantifying the shift in the community mean trait values due to environmental selection for the given functional traits (Ricotta & Moretti, 2011). All CWMs were computed with `functcomp` function in the R package “FD” version 1.0 (Laliberté & Legendre, 2010).

FDis, instead, reflect how much are functionally dissimilar species in a given assemblage:

$$FDis = \frac{\sum a_j z_j}{\sum a_j}$$

where a_j is the abundance of species j and z_j is the distance of species j to the weighted centroid (Laliberté and Legendre 2010). We selected the FDis index because it is not strongly influenced by outliers and by species richness. High/low values of FDis are expected to indicate a high/low degree of niche differentiation, *i.e.* functional divergence/convergence (Laliberté and Legendre, 2010). In computing FDis indices for all the three traits, (hereafter FDis_H, FDis_{SLA}), we considered gower distance as measure of traits dissimilarity (Pavoine et al., 2009). All the functional indices were computed with “`dbFD`” function in the R (2017) *FD* package version 1.0 (Laliberté et al., 2014).

Moreover, we analyse also variation over time in the taxonomic diversity and in the richness of dominant species. To achieve this task, we computed taxonomic diversity of dominant species using the following equation:

$$Rao = \sum_{ij}^n d_{ij} p_i p_j$$

where d_{ij} is the functional distance between species i and j and p_i are the relative abundances of the i -th and j -th species, and n is the number of species (Rao, 1982).

Taxonomic diversity of dominant species was calculated at plot level, considering only those species reaching the 80% of the total vegetation cover for each belt. At plot level, we calculated also the number of species considering only the presence of those species that contributed to reach the 80% of the total vegetation cover in each belt. For sake of brevity, we refer to “taxonomic diversity” and “species richness” in this study.

Species diversity indices were computed with the function “Rao” provide in the Supplementary Material in de Bello et al. (2010b).

Species diversity and functional structure variation over time

Shift in functional structure (CWM and FDis) over time were investigated performing analysis of the variance (ANOVAs). In each model, we used as response variables the functional indices (CWM and FDis) for single traits and as predictor the sampling event (2006, 2011 and 2016). In order to accommodate for functional structure variation characterizing each of the four section of the elevation gradient, we included as interaction term in the model the elevation belt. CWM for plant height and specific leaf area have been log-transformed to improve the assumption of normal distribution of the residuals.

Moreover, we performed analysis of the variance (ANOVAs) with interaction term, also for the indices of species diversity, i.e. species richness and taxonomic diversity. We log-transform this latter index, to improve the normality of the residuals.

All the analyses have been performed under R statistical environment (R Core Team, 2017) using “aov” function in “stats” package, and the assumptions of the linear model were evaluated by visually inspection checking for normality, homoscedasticity, and independence of the residuals.

RESULTS

Regarding plant height, we found a significant variation over time for FDis for the second belt (p-value: 0.03; Fig.2) and CWM for the third (p-value: 0.03) (Fig. 3). Concerning the specific leaf area, we found no significant change over time for CWM in any of the elevation section (Fig.4). Contrary, FDis in the second belt showed a significant variation over time (p-value: 0.03) (Fig.5).

Regarding species diversity, we found an increasing of species richness for each belt over time, with the exception of the lower section (Fig.6). While, for taxonomic diversity (Fig.7) we found a increasing trend over time in each of the elevation section.

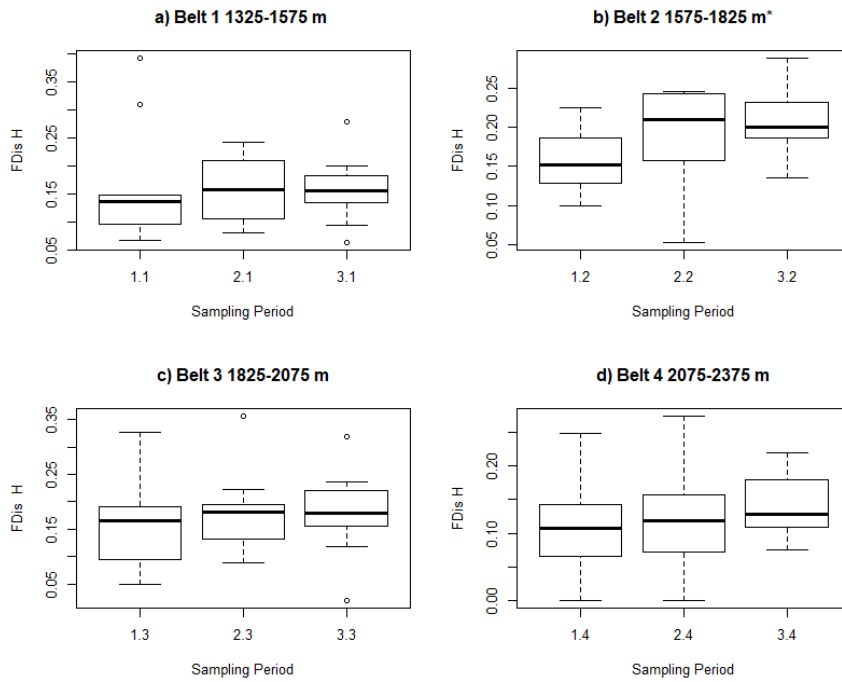


Fig.2. Variation of functional dispersion for plant height (FDIs_H) for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

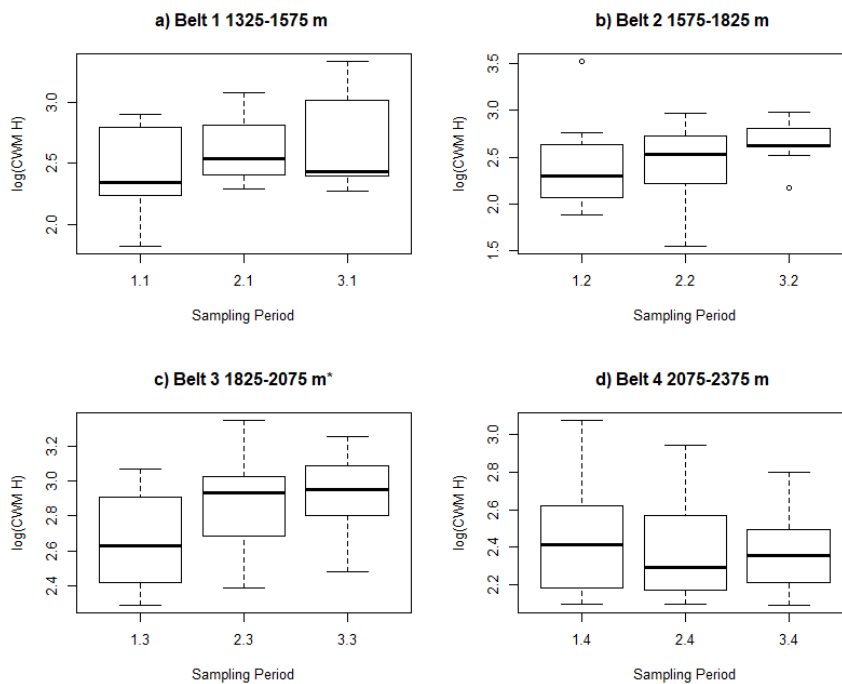


Fig.3. Variation of community-weighted mean for plant height (CWM_H) for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

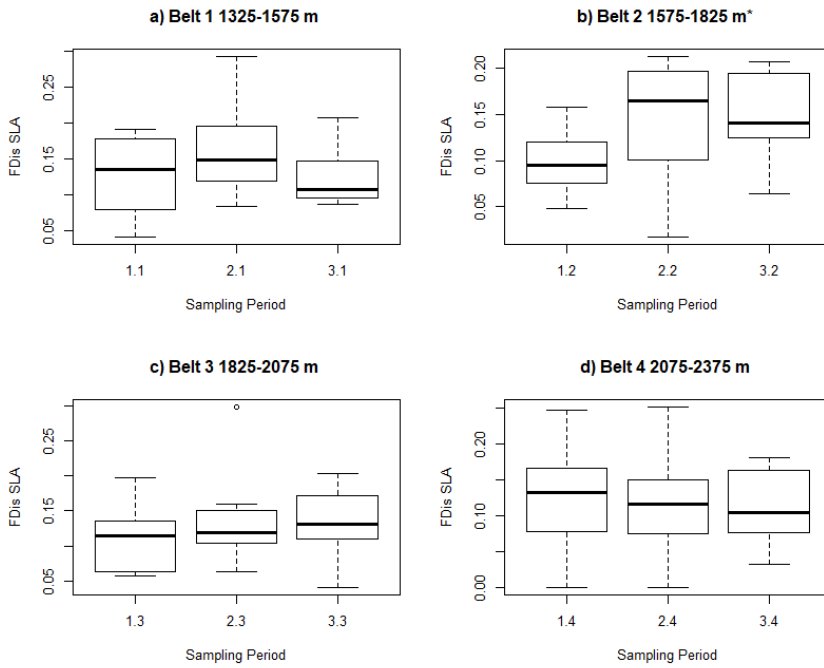


Fig.4. Variation of functional dispersion for specific leaf area (FDi_{SLA}) for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

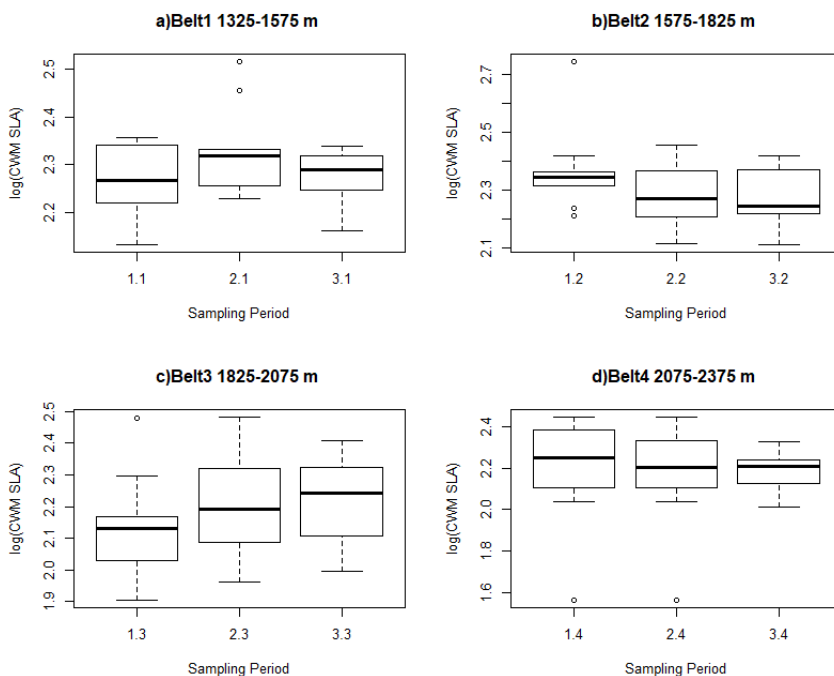


Fig.5. Variation of community-weighted mean for specific leaf area (CWM_{SLA}) for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

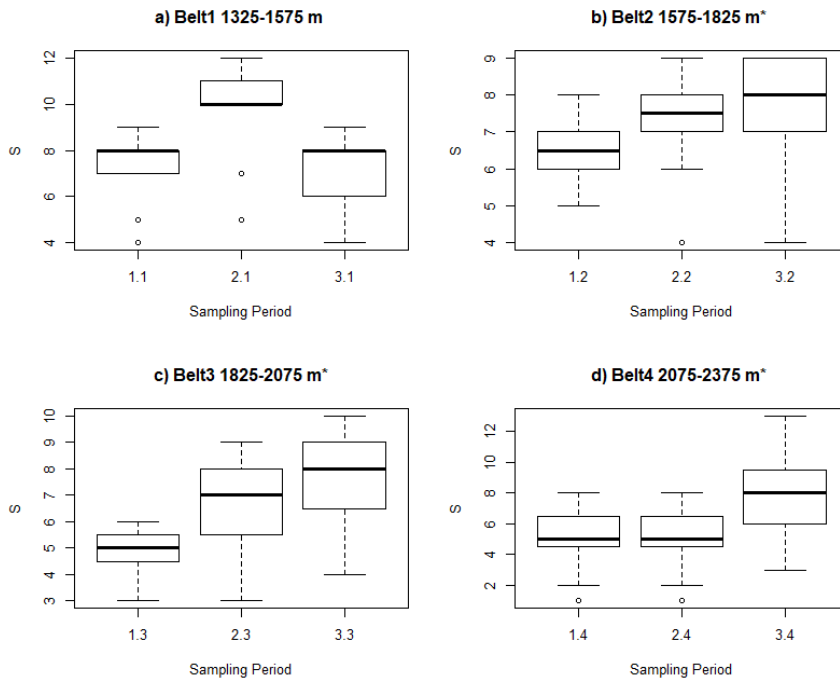


Fig.6. Variation in the number of species reaching the 80% of the total vegetation cover (S) for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

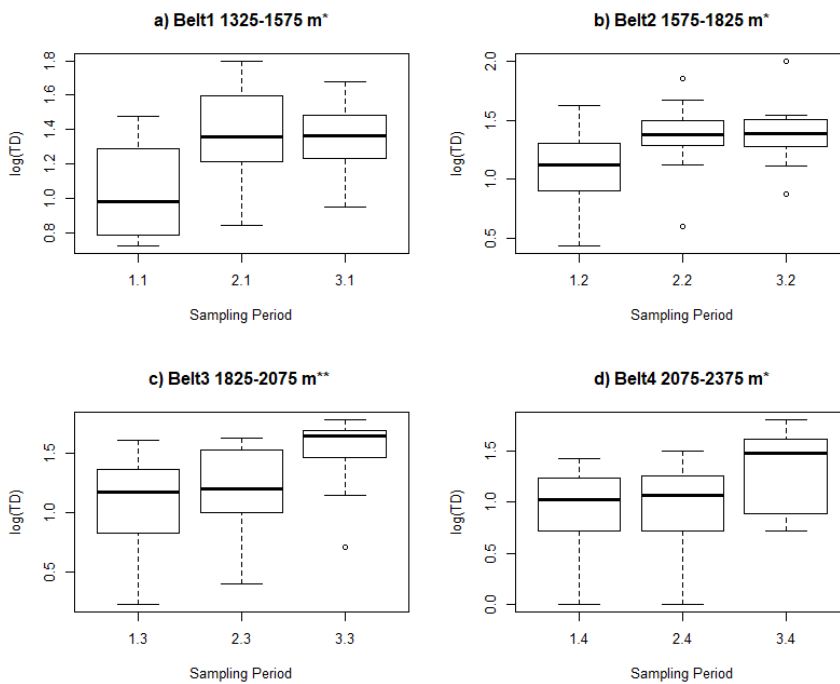


Fig.7. Taxonomic diversity (TD) variation considered only those species reaching the 80% of the total vegetation cover for grassland communities located on Velino Massif over time. Letter referred to single elevation section: first section a), second section b), third section c) and fourth d). Asterisk near the title of the graph is referred to pvalue (***, $P = 0.001$; **, $P < 0.01$; *, $P < 0.05$)

DISCUSSION

Our study highlighted that during the last decade important changes in species diversity and functional traits occurred in grasslands along the elevation gradient of Velino massif. These vegetation changes are in line with the dynamism detected for this particular area (Malavasi et al., 2018).

Interestingly, we found that species diversity and functional traits vary over time differently along the elevation gradient. The second and the third belt showed a higher functional variation over time, contrary, species diversity variation was observed for almost all the length of the gradient. This could suggest a higher functional redundancy for the lower and higher belt. Indeed, increasing species diversity within a narrow range of functional diversity resulted in fact in higher redundancy (de Bello et al., 2009).

Moreover, we found that lower functional redundancy was found in the middle part of the elevation gradient, where the environmental conditions were supposed to be less constrained (Table 1), suggesting that the ecological niche related to these traits are not yet saturated. This pattern seemed in line with previous suggestion that stated humid grassland lower functionally redundant with respect to arid grassland (de Bello et al., 2009).

Increasing of functional structure in the middle part of the gradient has an effect of the ecosystem services. Indeed both traits, plant height and specific leaf area, are related to a range of the ecosystem services (de Bello et al., 2010a). Plant height is associated to climatic regulation, thus an increasing in the mean and diversity of the height of community, may affect positively the ecosystem by increasing the carbon sequestration (de Bello et al., 2009). Otherwise, higher diversity of leaf strategies entails a higher diversity of photosynthetic and growth rates, leaf longevity, litter decomposability, etc... Hence, a higher SLA diversity may have a positive effect on ecosystem processes such as productivity and nutrient retention (Tilman et al., 1997).

Moreover, in more functionally redundant communities (low and higher elevation), a lower variability in ecosystem processes is expected (de Bello et al., 2009), resulting in a smaller variability in vegetation responses to the environment and in ecosystem processes with increasing diversity.

However, in this study we have to consider that the weak functional variation over time could be probably due to the temporal gradient that is not enough length. Long-term studies have found a stronger variation along wider gradient, i.e. more than two decades (Naaf & Wulf, 2011; Giarrizzo et al., 2016). Additionally, ten years may be not enough to provide a strong environmental changing for these grasslands. Species composition has been shown to play an important role in trait variation across communities distributed along steep spatial and temporal gradients (Kichenin et al. 2013; Lepš 2014). Contrary, intraspecific trait variation may played a dominant role along short spatial or temporal gradient, as it has been found under short-term management (Volf et al., 2016).

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CONCLUSION

There is an urgent need to improve our understanding of species coexist and how species are affected by global change, in particular for ecosystems like semi-natural grassland providing a wide range of ecosystem service. Detecting early signs of ecological change before abrupt shift occur, represent a hard but fundamental challenge for functional ecologist. It is more reliable that we are likely to close to win this challenge using multiple functional approach.

The general aims of my thesis were: 1) to provide an methodological approach to identify the processes behind the observed pattern; 2) to assess the relevance of intraspecific variation in functional ecology investigation; 3) to investigate belowground functional dimension comparing with the aboveground traits, proposing also a new traits related to new functional dimension; and 4) to assess the variation over time of species composition and functional traits, in relation to ecosystem services and functioning.

This thesis aimed to elucidate that predicting plant distribution needed to extend the currently research area, indeed the main findings are that 1) the metodological approach proposed to identify the assembly rules may be good but not for all traits; 2) the intraspecific trait variability is a fundamental component of trait variation at different level of biological organization; 3) taking into account few aboveground traits may not sufficient to understand how abiotic and biotic features affect species coexistence, thus belowground functional dimension needed a major investigation; 4) monitoring the ecosystem services and functioning may request a direct observation of an unified approach.

Here I briefly summarize the main findings for the four aspects covered in this thesis (assembly rules, intraspecific trait variability, belowground functional space and the use of trait in long-term studies):

1) Assembly Rules

In the Chapter 1 I proposed a methodological approach to disentangle the different processes that lead to the same functional pattern that I tested along elevation gradient on mediterranean grassland. The unimodal relationship between trait and environmental that I found, highlighted that trait-elevation relationship is climatic context dependent. While in temperate context, temperature seem to be the only limiting factor for plant performances at higher quota, promoting a linear decreasing, in submediterranean context plant communities seemed to be constrained at lower elevation by summer drought and higher elevation to winter frost, leading to the “hamp-shaped” model. Trait response was clear enough for plant height and seed mass, while specific leaf area showed high variation along the elevation gradient, thus likely other factors than climate may affect its functional pattern.

More important, our results highlighted that combining the mean and the dispersion of a given trait within the community may represent a useful approach to disentangle the processes only for traits related to competitive effects of the species. Plant height and seed mass, are traits related to the ability to suppress species (competitive effects or competition size-asymmetric): tall species may exclude short species, in the same way tall seedling may exclude the short seedling. The lack of relationship for specific leaf area, may be due to the

fact that this trait is related to competitive response and it is unaffected by processes like negative biotic interaction.

Disentangling assembly rules is still a challenge and our study represent an effort in this direction. Further investigation, along productive gradient using this unified approach may help to shed light on these results, providing more evidence about the lack of response for traits related to resource acquisition.

2) Relevance of Intraspecific trait variability

I investigated the relevance of intraspecific variation at two different scale: population level (Chapter 2) and community level (Chapter 3a).

At population level, I focused on the species *Brachypodium genuense*, in the mountain meadow of Central Apennine. *Brachypodium genuense* is a tall grass species able to spread in the plant community after cessation of traditional management, and causing a drop in the biodiversity. We investigate its relationship with different (micro, meso and macro) environmental factors. Contrary to suggestion asserting that plasticity is more affected by variation in the microenvironmental condition, we found an even contribute to intraspecific variability from microenvironmental condition as well as macroenvironmental condition. However, the variability of this is high and it ranges from competition performances (associated to high growth rate) in productive environment, to stress tolerant performance (associated to slow growth rate) in more unproductive environment. The ability of this species to spread and dominate community in different environmental condition may be due, not only to the clonal ability, but also to its ability to change its aboveground performances. Therefore management practices to keep under control its spread are necessary more in productive than in unproductive environment, due to high rate of spread under benign environmental condition. At community level (Chapter 3b) I focused on the same elevation gradient on Velino massif. I highlighted that the contribute of intraspecific trait variation to the community trait variation along the elevation is double than species composition for both traits, and that the general lack of the response for the aboveground traits is probably due to the negative covariation. Disentangling the contribute of these two effects, allow a better understanding of plant-environmental relationship, since some community pattern may mask from opposite trend of intraspecific trait variation and species composition. Moreover, along short gradient or within the same habitat, neglecting intraspecific trait variation may lead to misleading results, since it could be the main source of trait variation, with respect to long gradient that encompass different habitat, with different flora.

3) Belowground functional dimension

Still in Chapter 3a and in Chapter 3b, I investigated the belowground functional dimension comparing with the aboveground traits.

In particular, in Chapter 3a I proposed also a new belowground traits. I assessed its variation across environmental condition as well as with aboveground traits along the same gradient, both at species and community scale. We found contrasting pattern: at species level the lack of any trade-off between between this new trait with all the others, including the aboveground counterpart, suggest a new axis of plant performances

that this new trait is supposed to capture. At community level, we found a opposite pattern with respect to the aboveground counterpart, suggesting that these traits are shaped by different assembly processes. This new easy-to-measure trait may represent an extension of the plant functional strategy, and belowground resource conservation may represent an independent axis, whose investigation may improve our ability to predict species coexistence. However, measuring this trait in other habitat along different gradient, may help to confirm the results of this research.

I found the same opposite pattern also at a higher spatial scale, i.e. across habitat, with a collaboration with Institute of Botany of Academic Science of Czech Republic (Chapter 3b). This work adds new evidence on how it is fundamental investigating the belowground functional traits, since we found that abiotic condition act as a filter more on belowground traits than on aboveground. This is a further evidence of how belowground traits are fundamental in predicting plant response to environmental variation more than aboveground traits, at least at higher scale.

4) Species composition vs functional trait variation along temporal gradient: permanent plot-based approach. Finally, in the last Chapter of my thesis (4) I investigated the temporal shift in the species composition and functional traits along the elevation gradient on Velino massif. This was possible because since the 2005 permanent plots have been positioned and two previous sampling period (2006-2011) have been done. This work represents one of the firstly contribute to long-term observation for this study area. The results highlighted a different rate of changining with a higher rate of variation in the species diversity than in functional structure of the community. Combining the two measure allow us to investigate how ecosystem functioning and services may change. Indeed, we found that in more unproductive condition, increasing in species diversity and richness is not followed by a variation in the functional structure, while in the middle part at this increasing we found also an increasing of traits diversity. This highlighted that, even after land abandonment started almost 60 years ago, the pool of available niche is not yet saturated, increasing the ecosytem services and processes in this part of the gradient.

Is the relationship between trait dispersion and trait mean useful to reveal community assembly processes? A case study from sub-Mediterranean mountain grasslands

Alessandro Bricca¹, Luisa Conti², Maria Federico Tardella³, Andrea Catorci³, Marco Iocchi¹, Jean-Paul Theurillat⁴, Maurizio Cutini¹

¹Department of Science, University of Roma Tre, Rome, Italy;

²Department of Botany, Faculty of Sciences, University of South Bohemia, Na Zlate Stoce 1, CZ-370 05 České Budějovice, Czech Republic;

³School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy;

⁴Centre Alpien de Phytogéographie, Fondation J.-M. Aubert, Champex-Lac, Switzerland & Section of Biology, University of Geneva, Chambésy, Switzerland

Appendix 1 Number of total species found for each elevation belt. “Species measured” referred to those species reaching the 80% of the total vegetation cover of each elevation section

| | Section 1 (1325-1575 m a.s.l.) | Section 2 (1575-1825 m a.s.l.) | Section 3 (1825-2075 m a.s.l.) | Section 4 (2075-2375 m a.s.l.) |
|------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Total species | 90 | 79 | 78 | 84 |
| Species measured | 11 | 10 | 12 | 17 |

Appendix 2 List of species measured for each elevation section

| Species | Section 1 (1325-1575 m a.s.l.) | Section 2 (1575-1825 m a.s.l.) | Section 3 (1825-2075 m a.s.l.) | Section 4 (2075-2375 m a.s.l.) |
|------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| <i>Avenula Praetutiana</i> | | | | X |
| <i>Anthyllis Montana</i> | X | X | | X |
| <i>Anthyllis Vulneraria</i> | | | | X |
| <i>Brachypodium genuense</i> | | X | X | |
| <i>Brachypodium rupestre</i> | X | | | |
| <i>Bromus erectus</i> | X | X | X | X |
| <i>Carex humilis</i> | X | X | X | |
| <i>Carex kitaibeli</i> | | | X | X |
| <i>Carex macrolepis</i> | X | | X | |
| <i>Cerastium tomentosum</i> | | | | X |
| <i>Cytisus spinescens</i> | X | X | X | |
| <i>Festuca ovina</i> | X | | | |

| | | | | |
|--------------------------|---|---|---|---|
| Festuca stricta | | | X | |
| Festuca violacea | | | | X |
| Globularia meridionalis | X | X | X | X |
| Helianthemum nummularium | | | X | X |
| Helianthemum oelandicum | X | X | X | X |
| Pedicularis elegans | | | | X |
| Polygala major | | X | | |
| Potentilla apennina | | | | X |
| Potentilla crantzi | | | | X |
| Sesleria Juncifolia | X | X | X | X |
| Sesleria nitida | | | X | X |
| Thymus praecox | | | | X |
| Thymus striatus | X | X | | |
| Valeriana Montana | | | | X |

Appendix 3 Results of generalized least square models for species richness (S) and for all functional indices. The nature of the relationship between species richness and functional indices with the elevation, the appropriate variance structure, coefficient values of intercept and AIC values have been reported. ns: not significant; * pvalue<0.05; ** pvalue <0.01; ***pvalue <0.001

| Index | Model | Variance structure | Coefficient value | AIC |
|---------------------|-----------|-----------------------------------|-------------------|--------|
| S | Linear | fixed weights | -0.001*** | 31.14 |
| FDis _{LHS} | Quadratic | fixed weights | -0.14*** | -114 |
| CWM _H | Quadratic | Exponential of variance covariate | -1.01*** | 21.60 |
| CWM _{SM} | Quadratic | Exponential of variance covariate | -1.08*** | -50.53 |
| CWM _{SLA} | Linear | fixed weights | -0.001* | -53.68 |
| FDis _H | Quadratic | fixed weights | -0.13* | -110 |
| FDis _{SM} | Quadratic | Power of variance covariate | -0.18** | -111 |
| FDis _{SLA} | Quadratic | fixed weights | -0.09* | -126 |

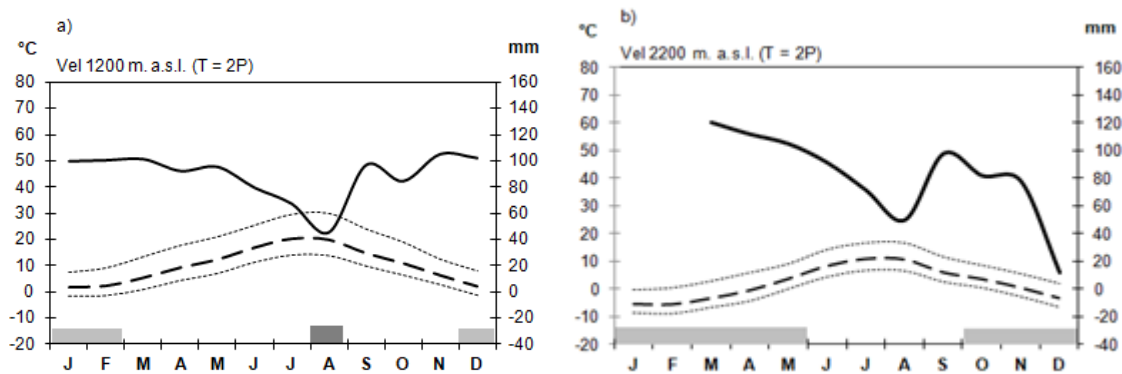
Belowground organ dry matter content: contrasting patterns of trait variation at species and community level

Alessandro Bricca¹, Gianluigi Ottaviani², Lars Götzenberger², Francesco de Bello², Maurizio Cutini¹, Jitka Klimešová²

¹Department of Science, University of Roma Tre, Rome, Italy;

²Institute of Botany, Czech Academy of Sciences, Dukelská 135, 37982 Třeboň, Czech Republic

Appendix 1 Lowest (a) and highest (b) termopluviometric stations present in the study area (Mt Velino Massif, Central Italy).



Appendix 2 List of species measured for each elevation belt and number of individual measured for the belowground organ dry matter content for each species in each elevation section.

| Species | Section 1 (1325-1575 m a.s.l.) | Section 2 (1575-1825 m a.s.l.) | Section 3 (1825-2075 m a.s.l.) | Section 4 (2075-2375 m a.s.l.) | N individual |
|---------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|-----------------|
| <i>Avenula praetutiana</i> | | | | X | 4 |
| <i>Anthyllis Montana</i> | X | X | | X | 2 |
| <i>Anthyllis vulneraria</i> | | | | X | 2 |
| <i>Brachypodium genuense</i> | | X | X | | 4 |
| <i>Brachypodium rupestre</i> | X | | | | 4 |
| <i>Bromus erectus</i> | X | X | X | X | 4 |
| <i>Carex humilis</i> | X | X | X | | 4 |
| <i>Carex kitaibeli</i> | | | X | X | 4 |
| <i>Carex macrolepis</i> | X | | X | | 4 |
| <i>Cerastium tomentosum</i> | | | | X | 4 |
| <i>Cytisus spinescens</i> | X | X | X | | 2 |
| <i>Festuca ovina</i> | X | | | | 4 |
| <i>Festuca stricta</i> | | | X | | 4 |
| <i>Festuca violacea</i> | | | | X | 4 |
| <i>Globularia meridionalis</i> | X | X | X | X | 2 |
| <i>Helianthemum nummularium</i> | | | X | X | 4 |
| <i>Helianthemum oelandicum</i> | X | X | X | X | 4 |

| | | | | | |
|---------------------|---|---|---|---|---|
| Pedicularis elegans | | | | X | 4 |
| Polygala major | | X | | | 2 |
| Potentilla apennina | | | | X | 2 |
| Potentilla crantzii | | | | X | 2 |
| Sesleria juncifolia | X | X | X | X | 4 |
| Sesleria nitida | | | X | X | 4 |
| Thymus praecox | | | | X | 4 |
| Thymus striatus | X | X | | | 2 |
| Valeriana Montana | | | | X | 4 |

Appendix 3 Trait scores for the three principal component axes.

| | PC1 | PC2 | PC3 |
|-------|--------|---------|---------|
| H | 1.5849 | -0.2489 | -0.3692 |
| SLA | 0.0932 | -1.3532 | 0.6146 |
| SM | 0.7653 | -1.2316 | -0.2761 |
| BODMC | 0.6627 | 0.3820 | 1.5334 |
| LDMC | 1.3421 | 0.9016 | -0.2064 |

Appendix 4 Results of generalized least square models (GLSs) for functional dispersion and community weighted mean of belowground organ dry matter content and leaf dry matter content. The appropriate variance structure, coefficient values of slope and AIC values have been reported. ns: not significant; * pvalue<0.05; ** pvalue <0.01; ***pvalue <0.001

| Index | Model | Variance structure | Coefficient value | AIC quadratic model | AIC linear model |
|-----------------------|-----------|--------------------|-------------------|---------------------|------------------|
| CWM _{BODMC} | Quadratic | fixed weights | -0.18* | -90.92 | -75 |
| CWM _{LDMC} | - | - | - | - | - |
| FDis _{BODMC} | Quadratic | fixed weights | 0.15*** | -150 | -125 |
| FDis _{LDMC} | Quadratic | fixed weights | -0.14*** | -136 | -117 |

Clonal vs leaf-height-seed (LHS) traits: Which are filtered more strongly across habitats?

Anna E. Vojtkó^{1,2}, Martin Freitag³, Alessandro Bricca⁴, Felipe Martello⁵, Joaquín Moreno Compañ⁶, Martin Küttim⁷, Robert Kun⁸, Francesco de Bello^{2,9}, Jitka Klimešová⁹, Lars Götzenberger⁹

¹MTA Centre for Ecological Research, DRI, Department of Tisza Research, Bem tér 18/C, H-4026 Debrecen, Hungary

²Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic

³Biodiversity and Ecosystem Research Group, Institute of Landscape Ecology, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany

⁴Department of Science, University of Roma Tre, V. le Marconi 446, Rome - 00144, Italy

⁵Spatial Ecology and Conservation Laboratory, Department of Ecology, São Paulo State University – UNESP, Av. 24A, 1515, Rio Claro, Brazil

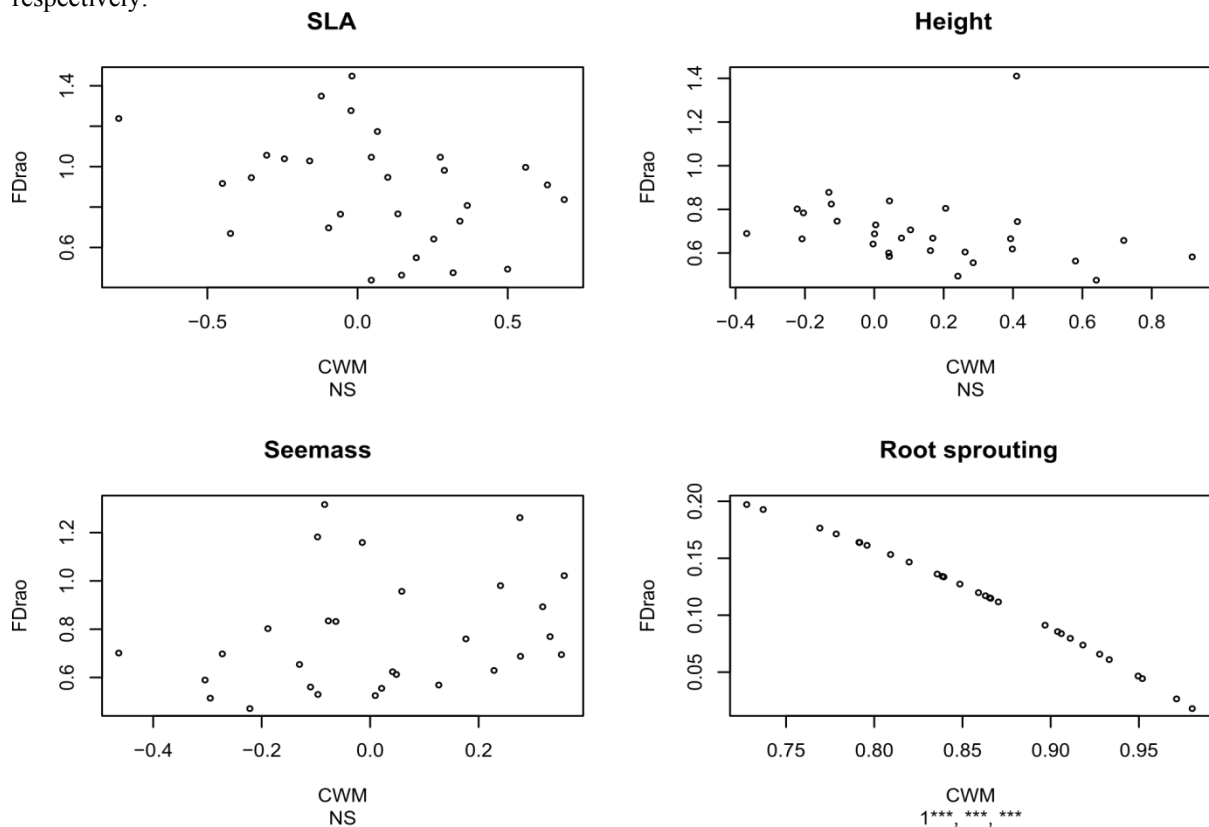
⁶Department of Environmental Sciences and Natural Resources & Institute of Biodiversity CIBIO, University of Alicante, Carretera de San Vicente s/n, 03690 San Vicente del Raspeig, Alicante, Spain

⁷Institute of Ecology, School of Natural Sciences and Health, Tallinn University, Uus-Sadama 5, 10120 Tallinn, Estonia.

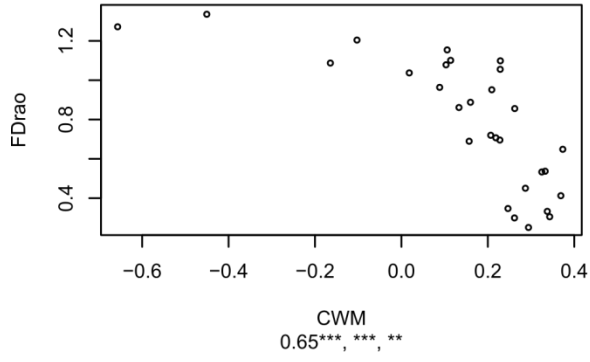
⁸Department of Nature Conservation and Landscape Ecology, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter Károly utca 1., 2103 Gödöllő, Hungary

⁹Institute of Botany, Czech Academy of Sciences, Dukelská 135, 37982 Třeboň, Czech Republic

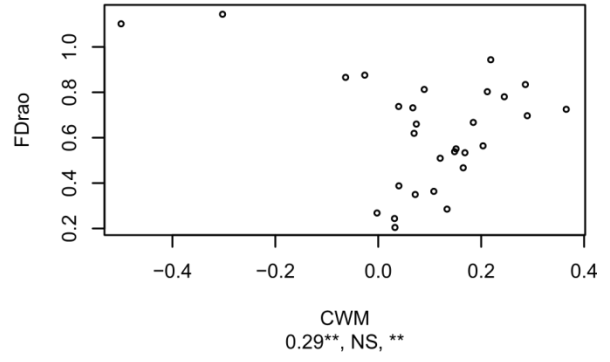
Fig. S1 Figures depicting the relationship between FD and CWM for single traits. The three terms under each figure represent the R^2 of the model and its significance, and the significance of the linear and of the quadratic terms, respectively.



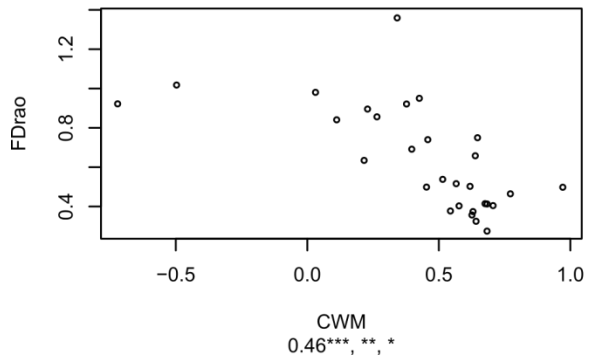
Budbank depth



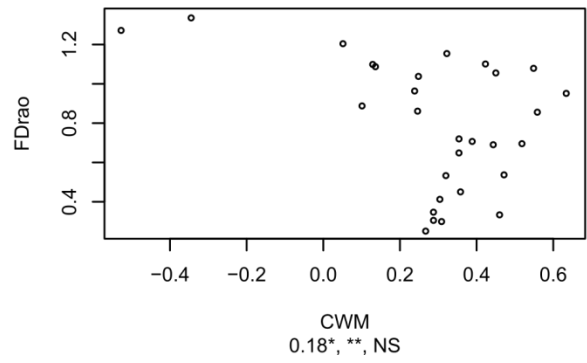
Budbank depth including root taps



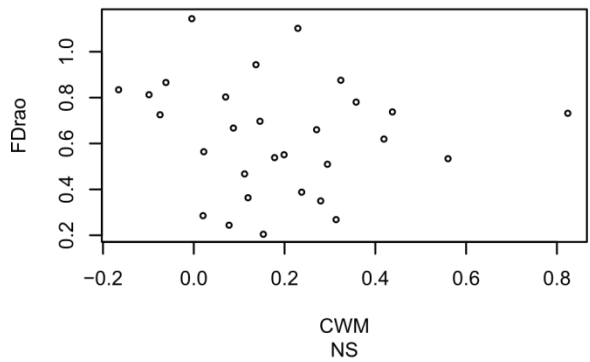
Budbank size



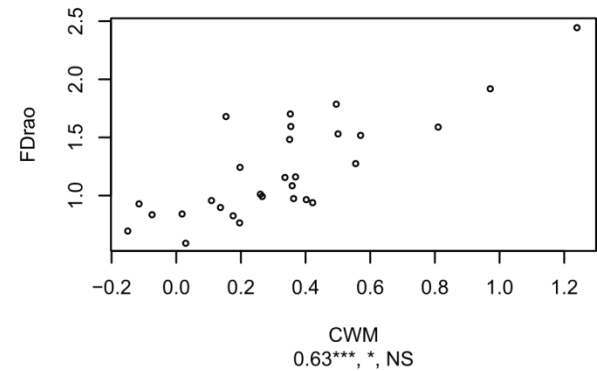
Budbank size including root taps



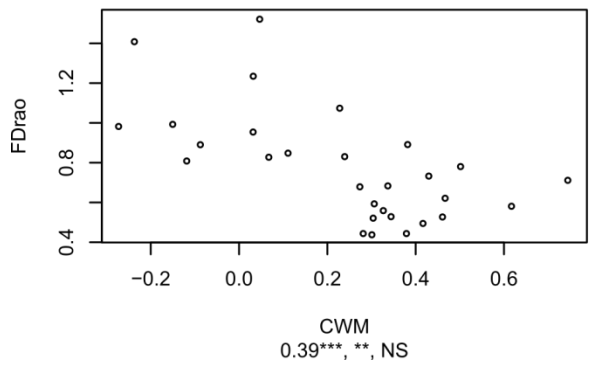
Multiplication rate



Lateral Spread



Clonal Index



Clonal vs leaf-height-seed (LHS) traits: Which are filtered more strongly across habitats?

Anna E. Vojtkó^{1,2}, Martin Freitag³, Alessandro Bricca⁴, Felipe Martello⁵, Joaquín Moreno Compañ⁶, Martin Küttim⁷, Robert Kun⁸, Francesco de Bello^{2,9}, Jitka Klimešová⁹, Lars Götzenberger⁹

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⁶Department of Environmental Sciences and Natural Resources & Institute of Biodiversity CIBIO, University of Alicante, Carretera de San Vicente s/n, 03690 San Vicente del Raspeig, Alicante, Spain

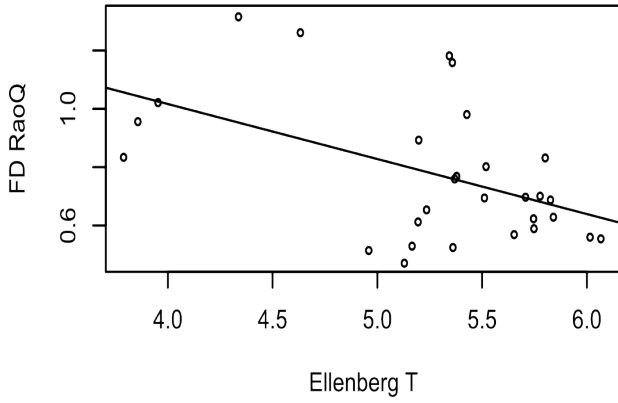
⁷Institute of Ecology, School of Natural Sciences and Health, Tallinn University, Uus-Sadama 5, 10120 Tallinn, Estonia.

⁸Department of Nature Conservation and Landscape Ecology, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter Károly utca 1., 2103 Gödöllő, Hungary

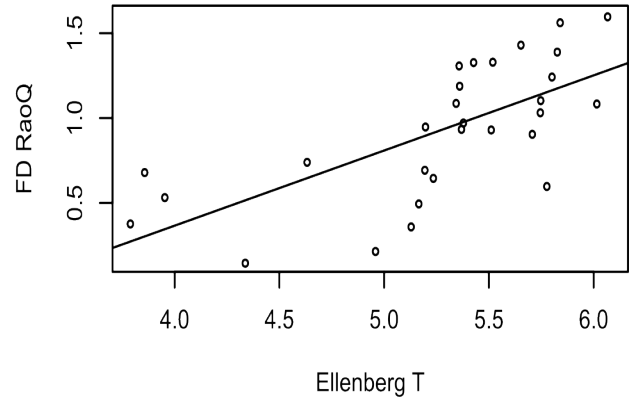
⁹Institute of Botany, Czech Academy of Sciences, Dukelská 135, 37982 Třeboň, Czech Republic

Fig. S1 (starting on following page) Figures depicting the relationship between FD and CWM, and single Ellenberg indicator values. Each relationship was modelled as a linear model with the Ellenberg value and its quadratic term as predictors. Figures are only shown for models that were significant for the linear term, the quadratic term, or both. The scatterplots are accompanied by the linear and quadratic lines, depending on their significance in the models.

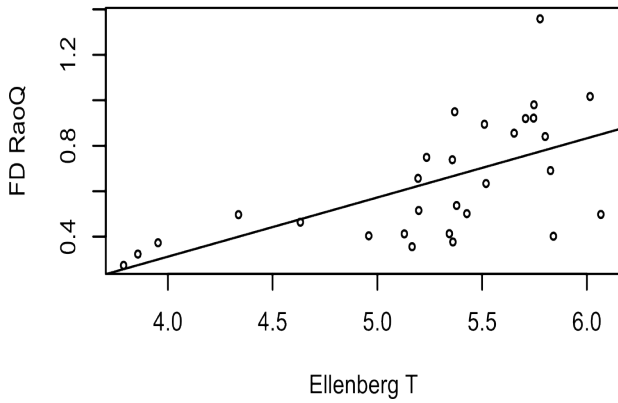
Seed mass



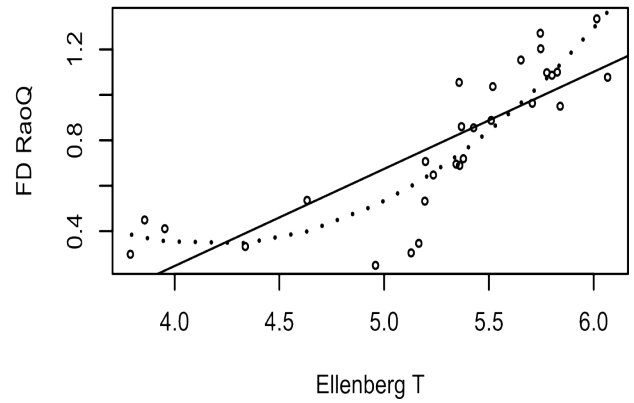
Root sprouting capacity



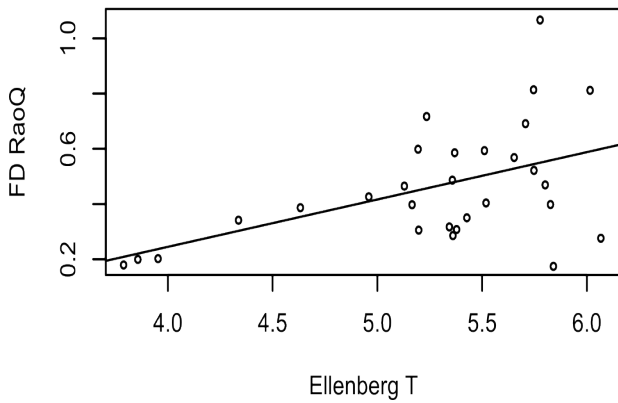
Bud bank size



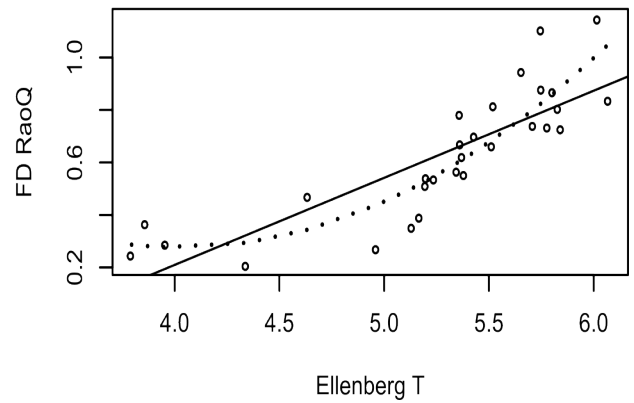
Bud bank size including root taps



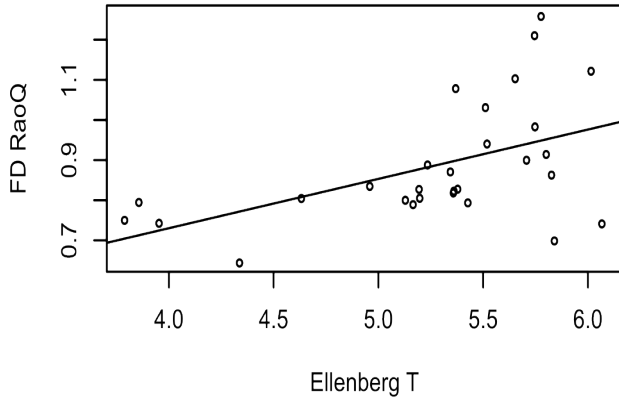
Bud bank depth



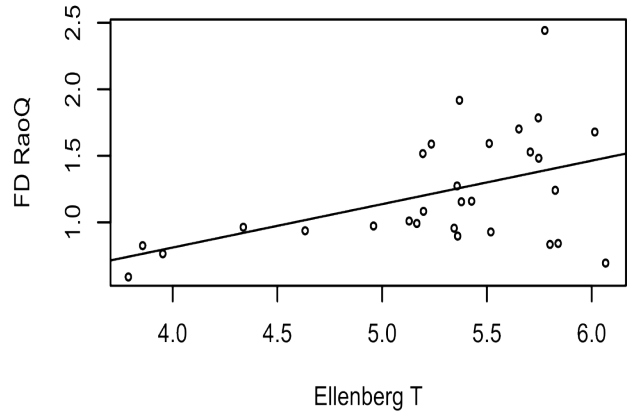
Bud bank depth including root taps



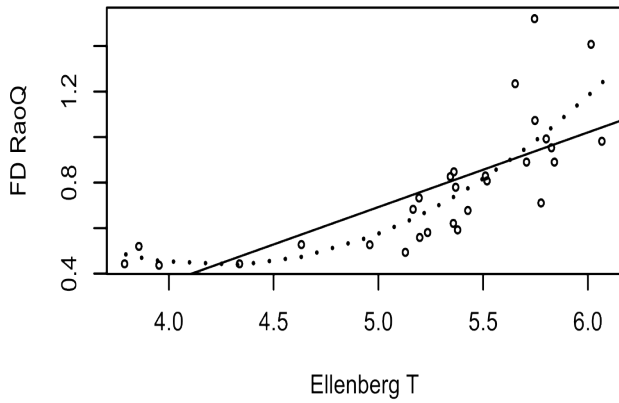
All bud bank traits



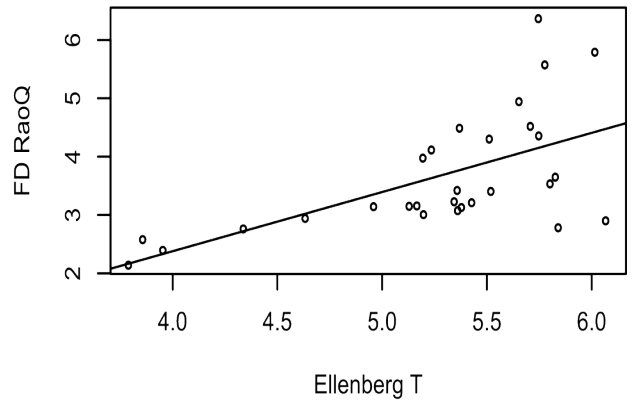
Multiplication rate



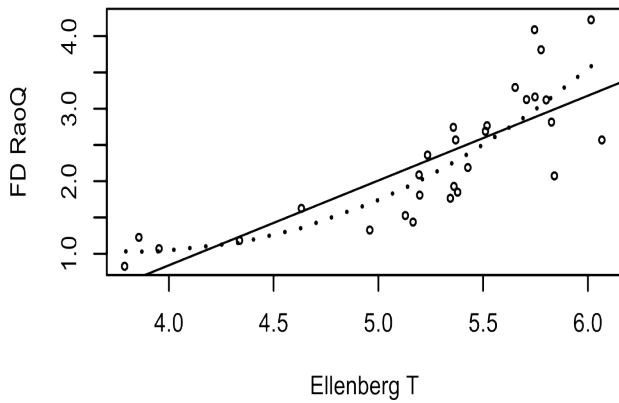
Lateral spread



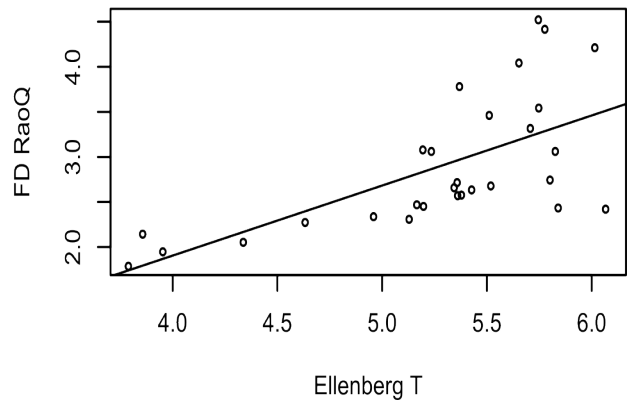
Clonal index



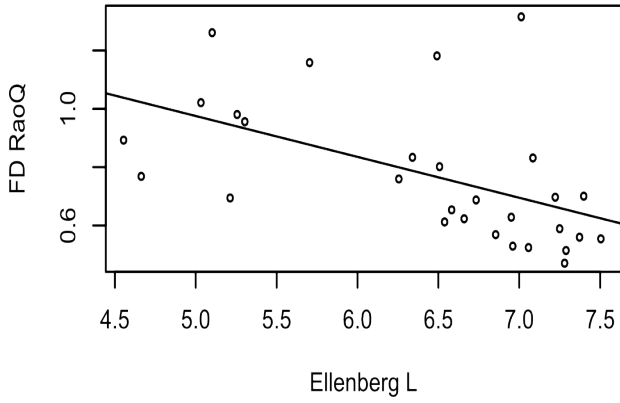
All Clonal traits



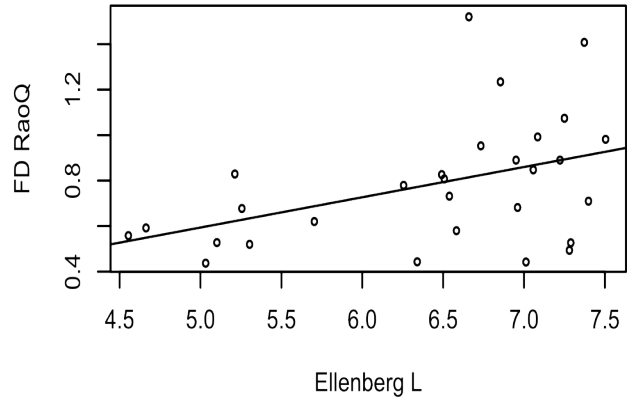
Clonal and bud bank traits



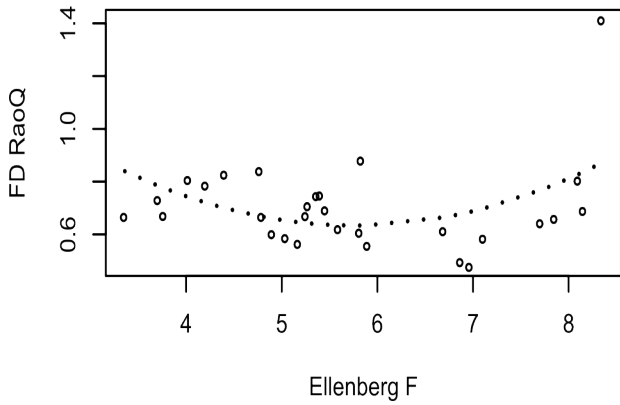
Seed mass



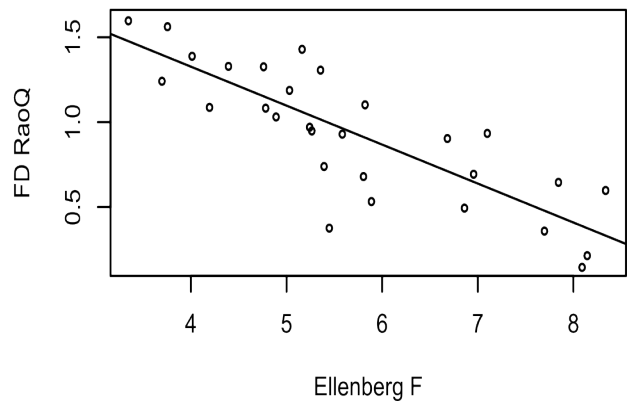
Lateral spread



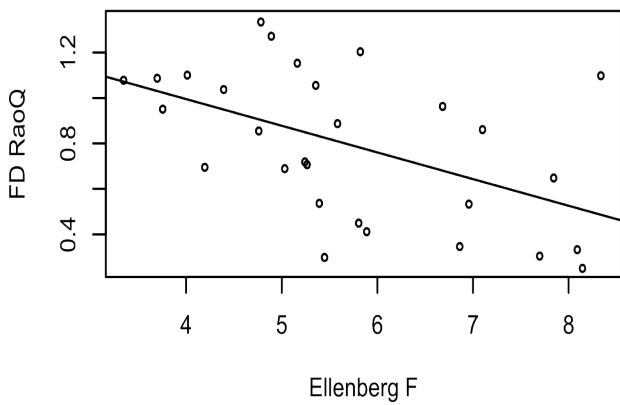
Height



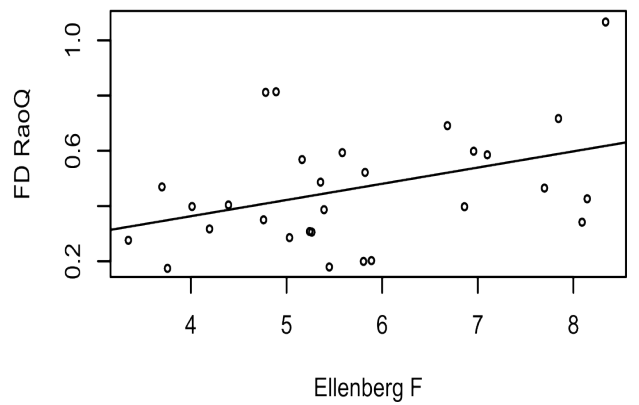
Root sprouting capacity



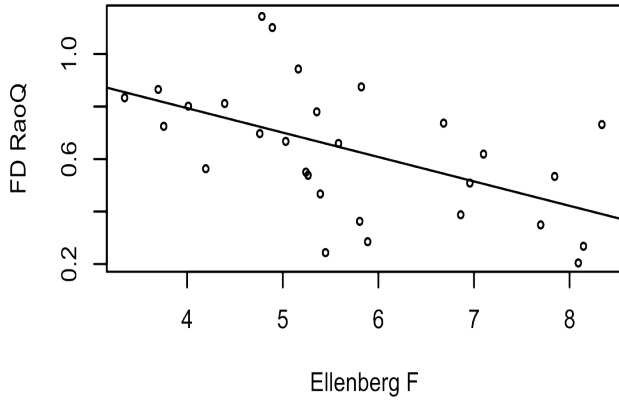
Bud bank size including root taps



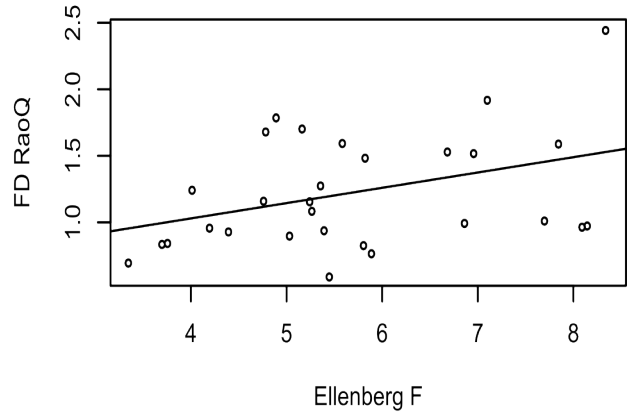
Bud bank depth



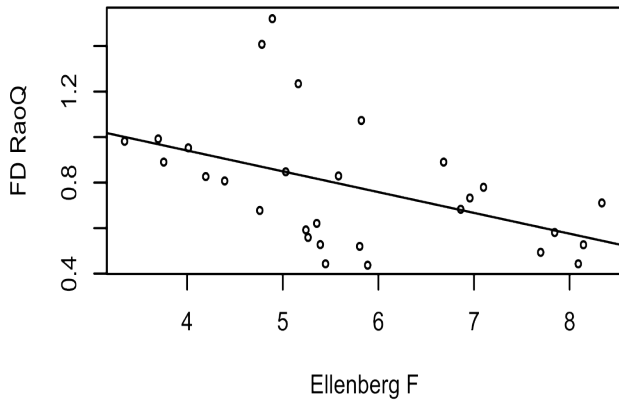
Bud bank depth including root taps



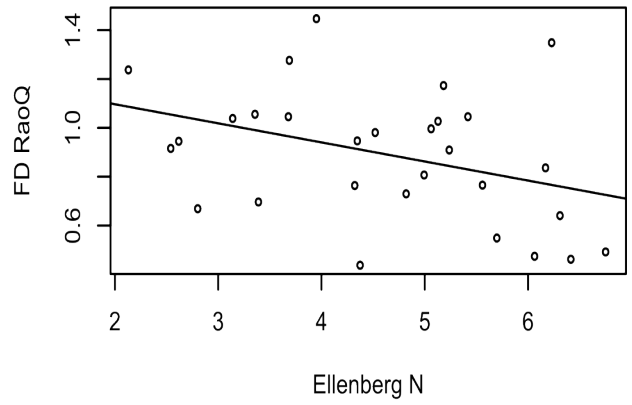
Multiplication rate



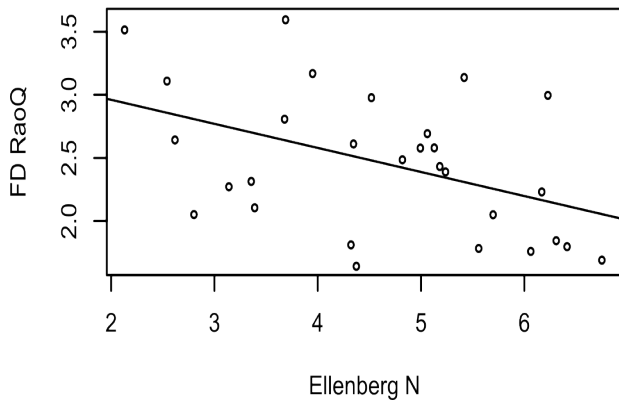
Lateral spread



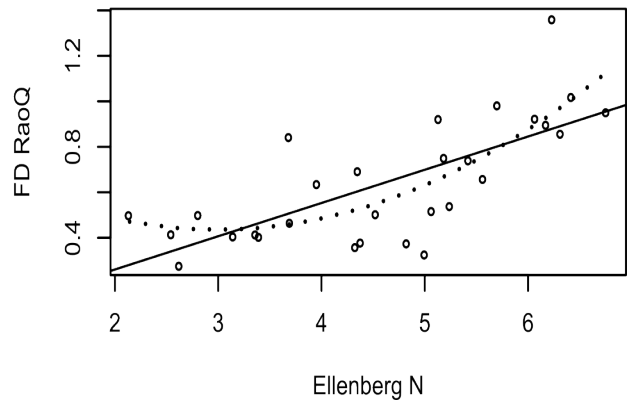
Specific leaf area



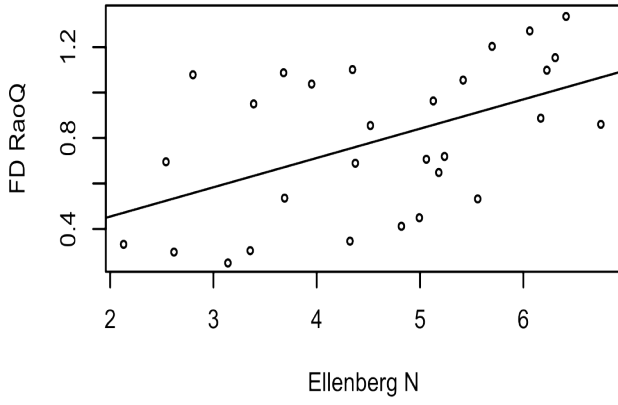
LHS traits



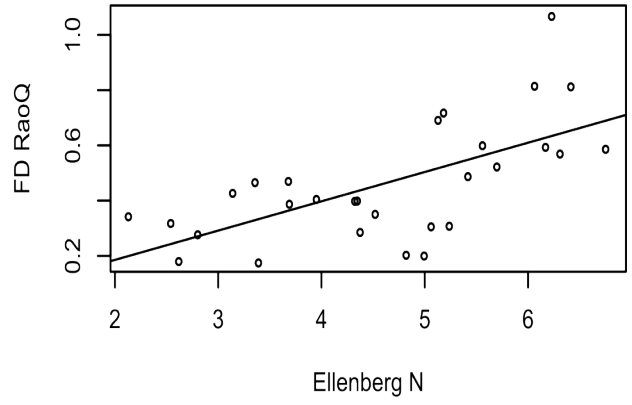
Bud bank size



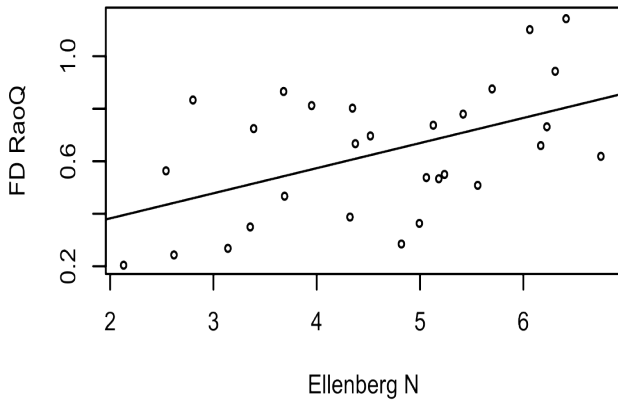
Bud bank size including root taps



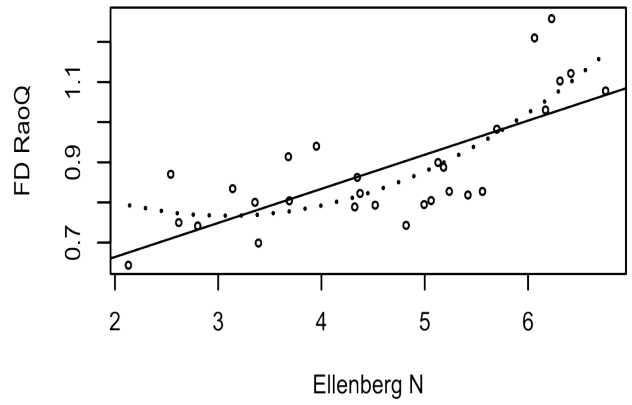
Bud bank depth



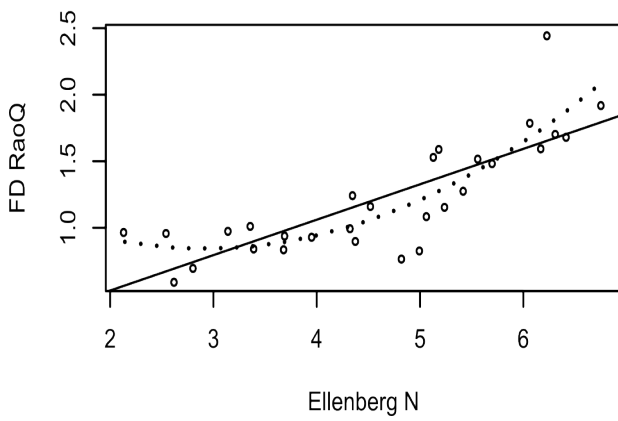
Bud bank depth including root taps



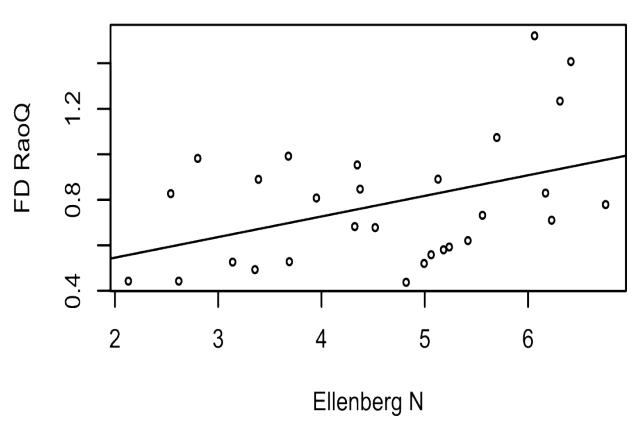
All bud bank traits



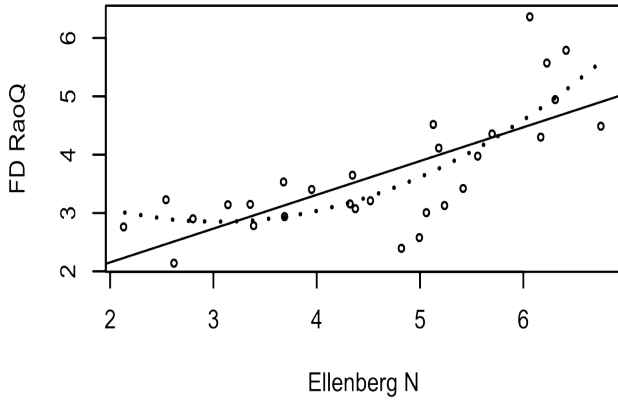
Multiplication rate



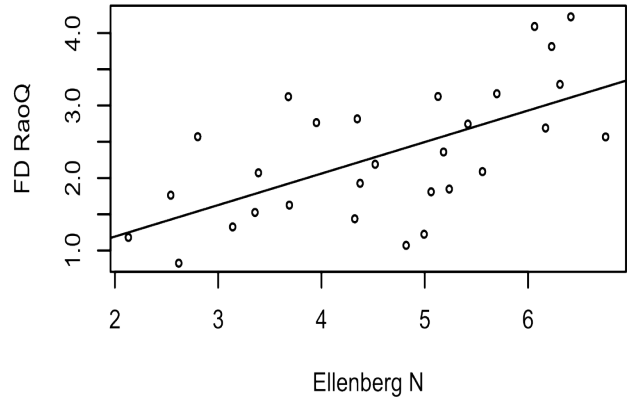
Lateral spread



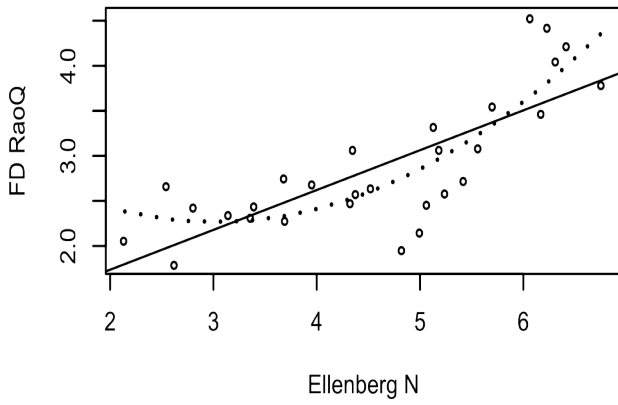
Clonal index



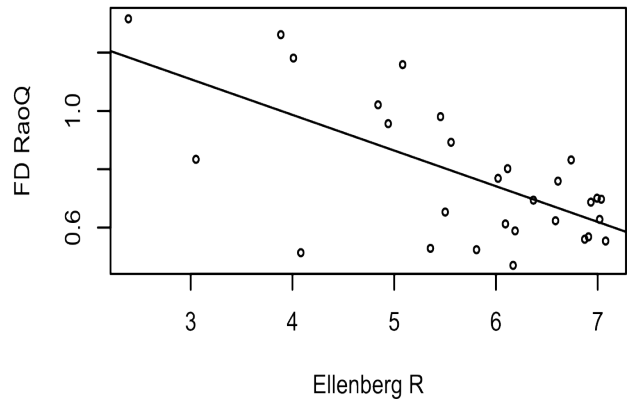
All Clonal traits



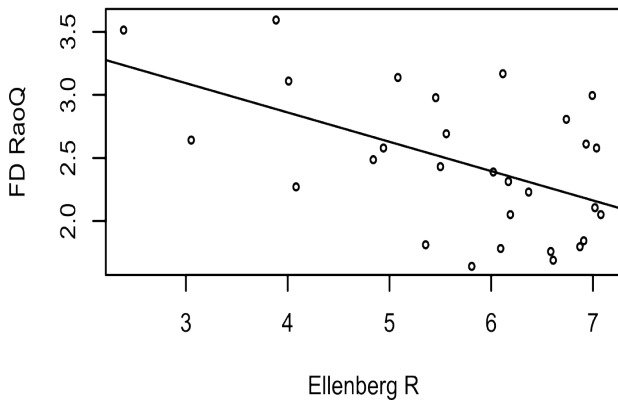
Clonal and bud bank traits



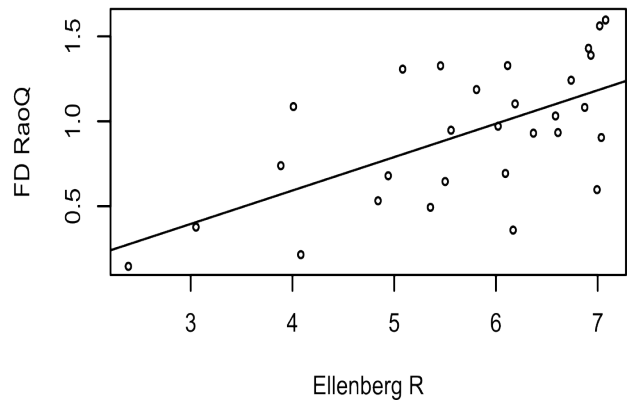
Seed mass



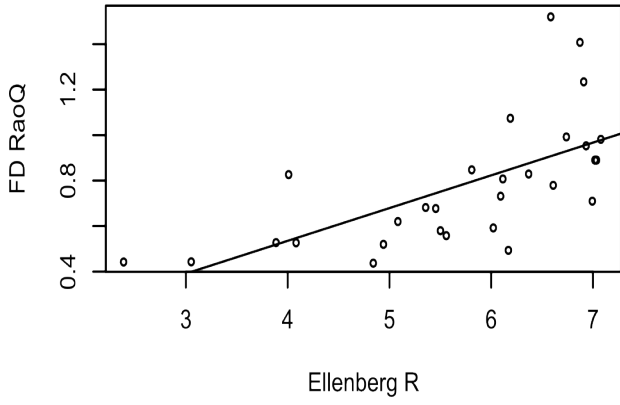
LHS traits



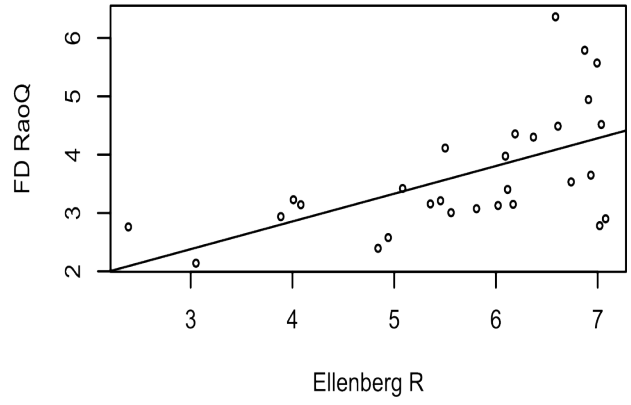
Root sprouting capacity



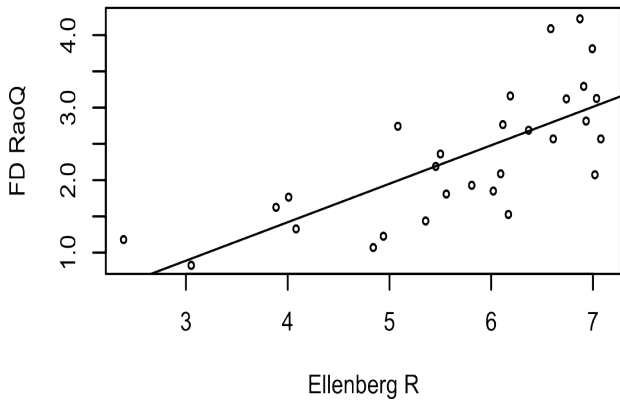
Lateral spread



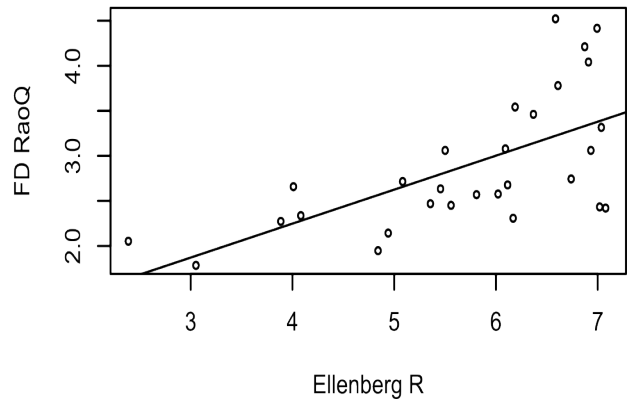
Clonal index



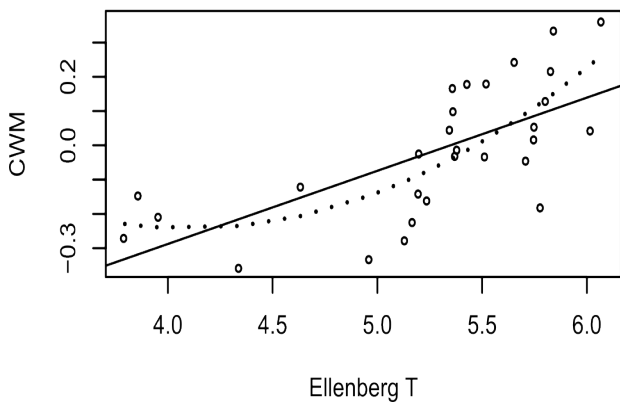
All Clonal traits



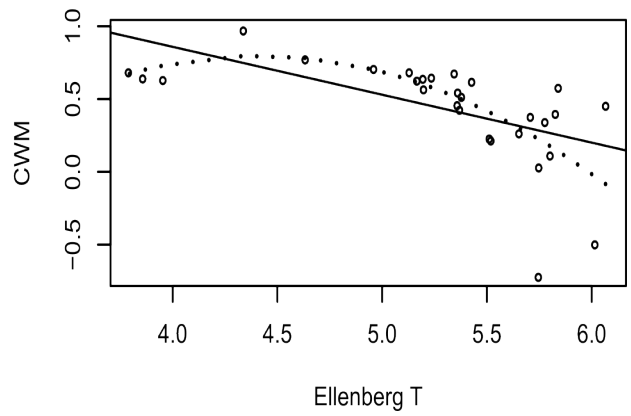
Clonal and bud bank traits



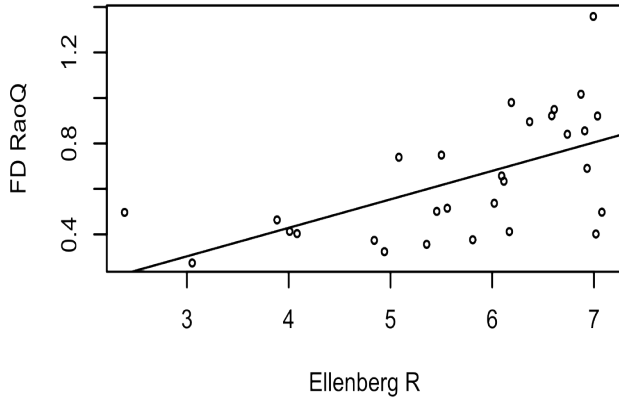
Root sprouting capacity



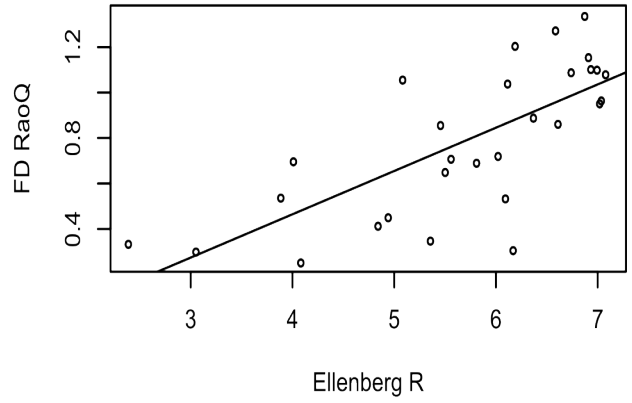
Bud bank size



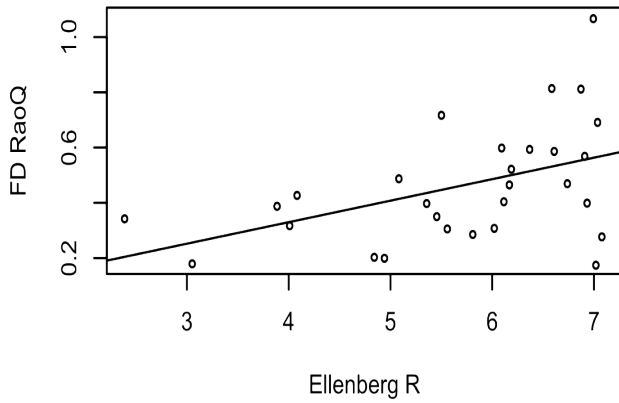
Bud bank size



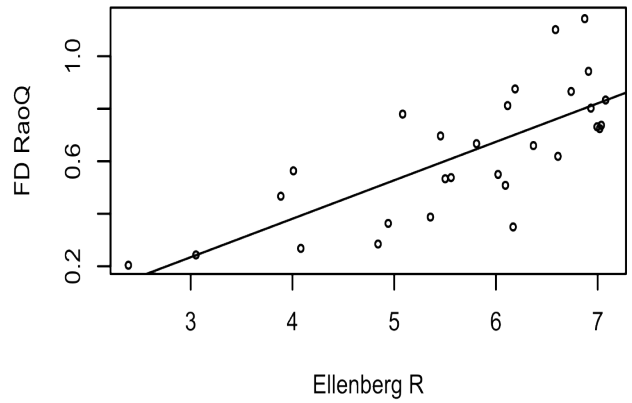
Bud bank size including root taps



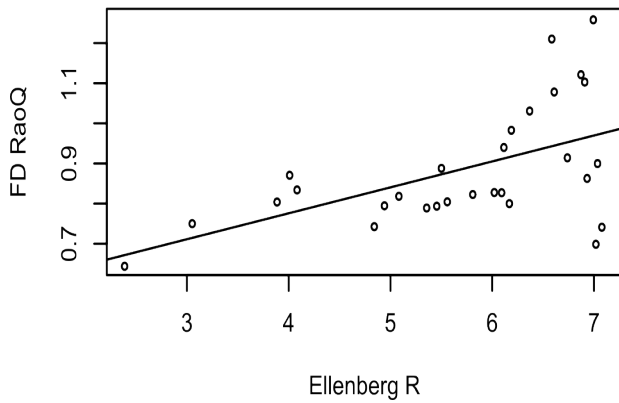
Bud bank depth



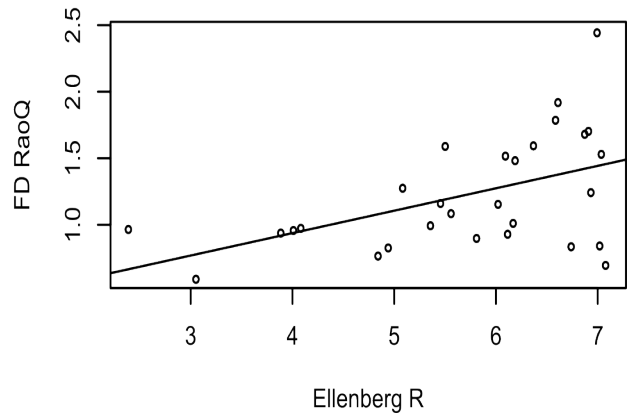
Bud bank depth including root taps



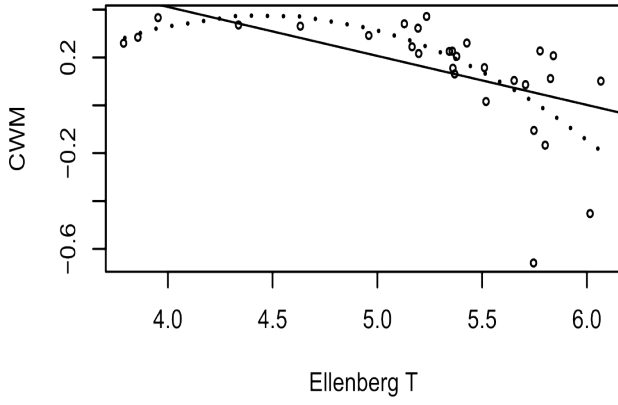
All bud bank traits



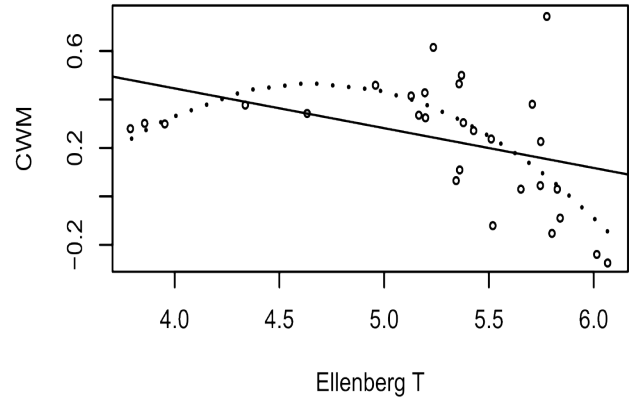
Multiplication rate



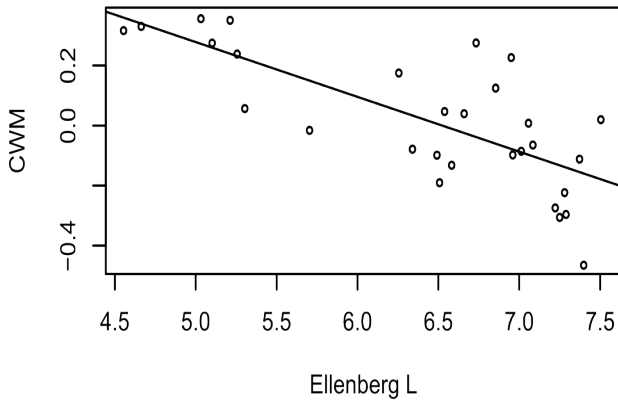
Bud bank depth



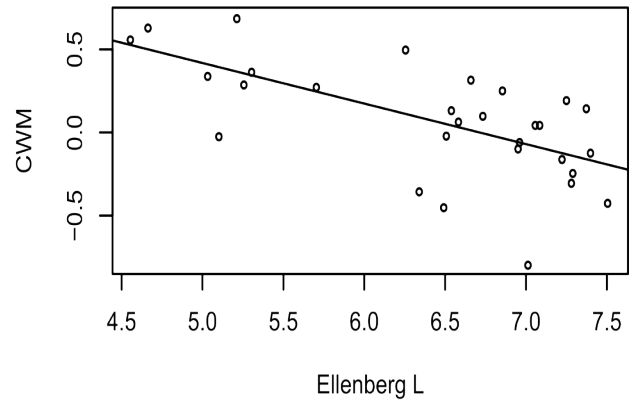
Lateral spread



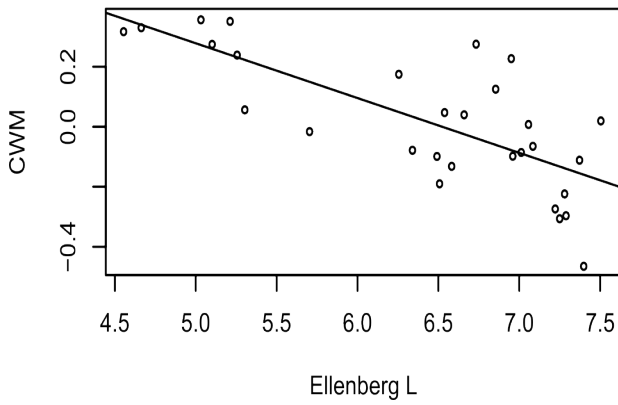
Seed mass



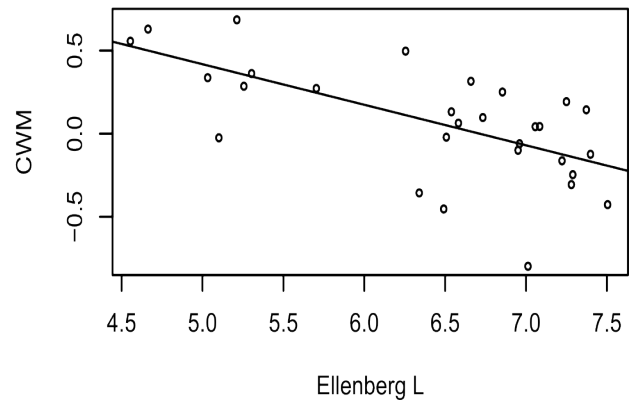
Specific leaf area



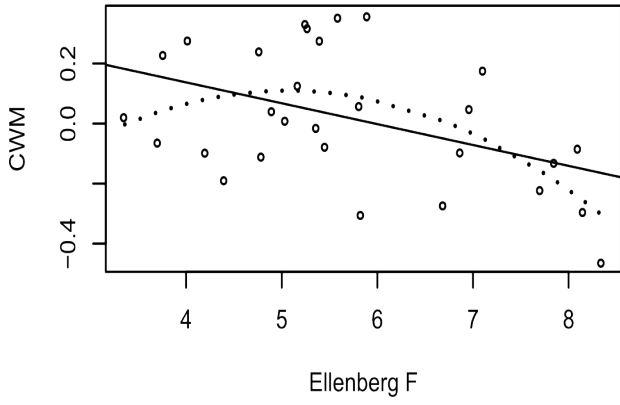
Clonal index



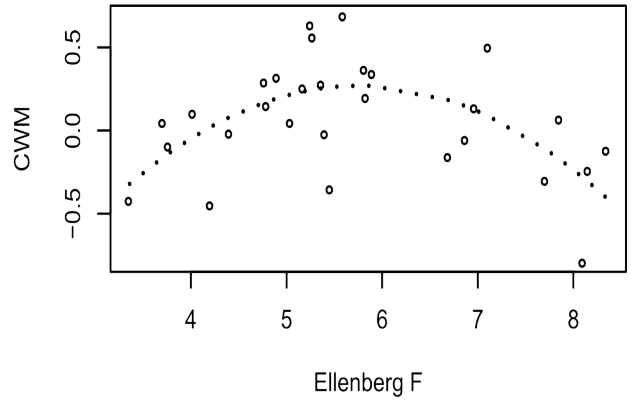
All Clonal traits



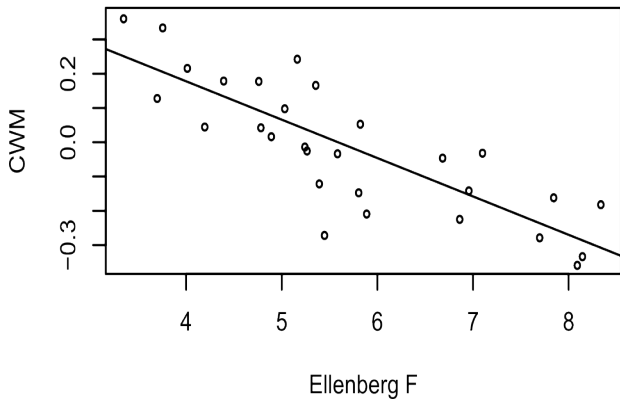
Seed mass



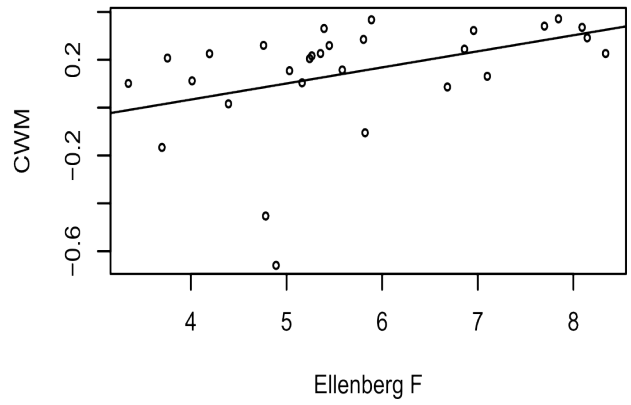
Specific leaf area



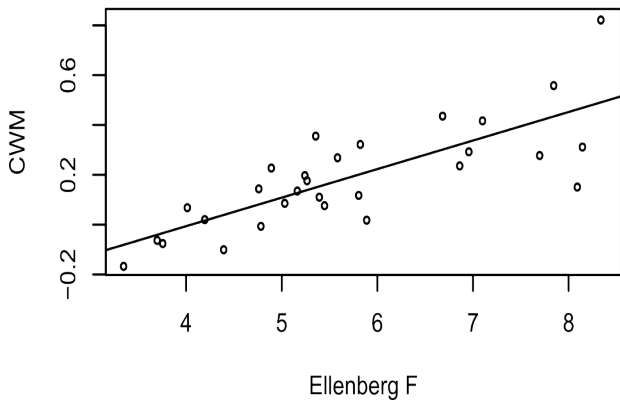
Root sprouting capacity



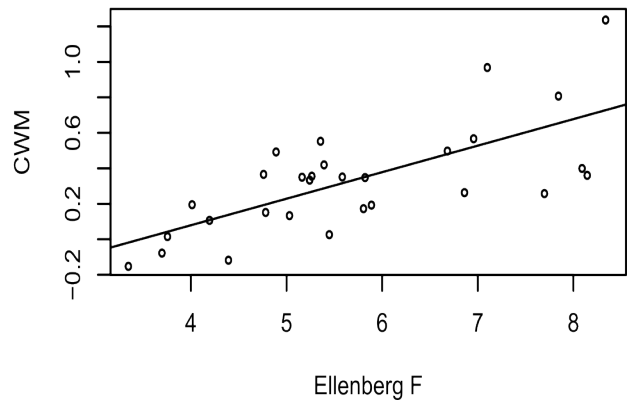
Bud bank depth



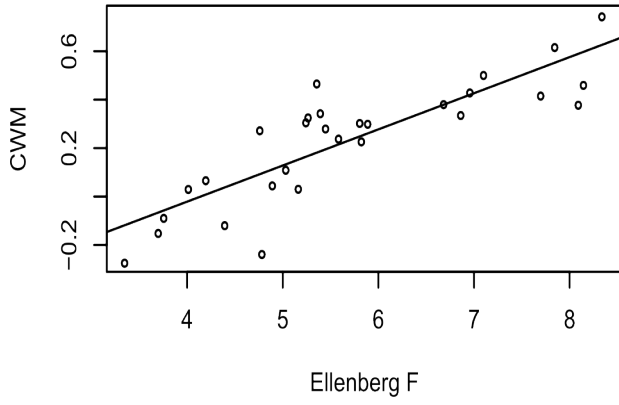
All bud bank traits



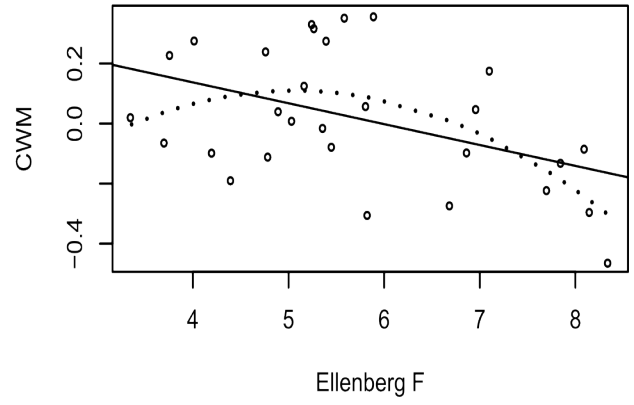
Multiplication rate



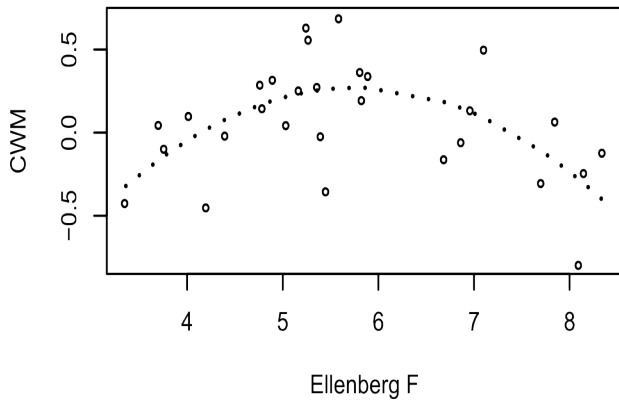
Lateral spread



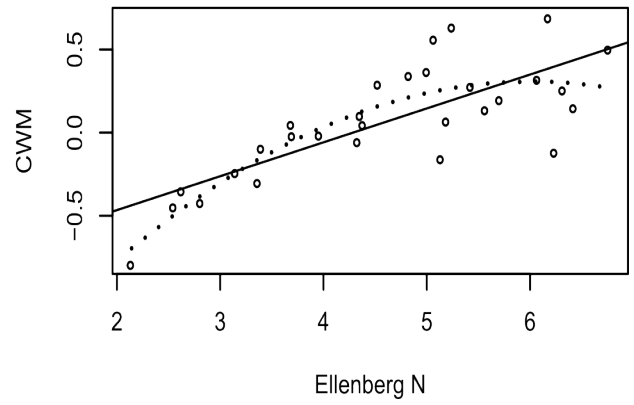
Clonal index



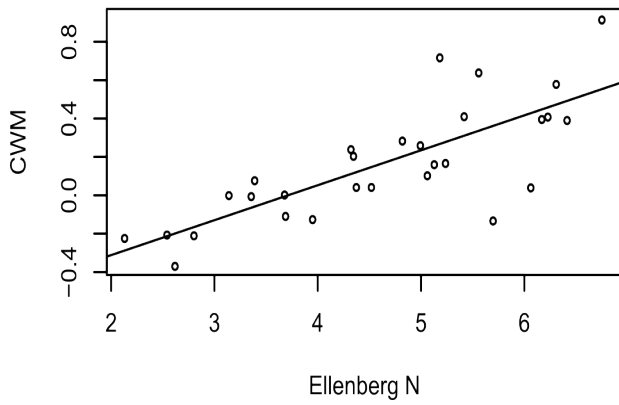
All Clonal traits



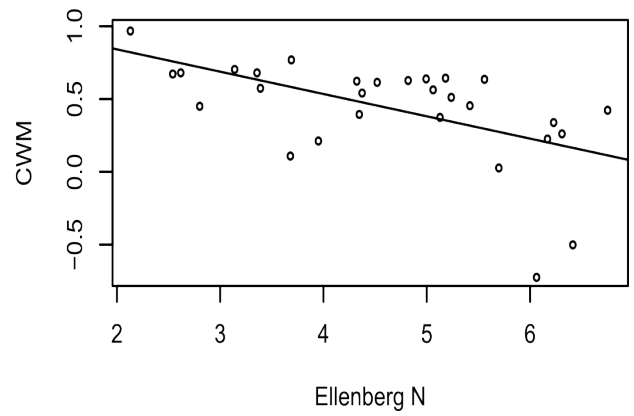
Specific leaf area



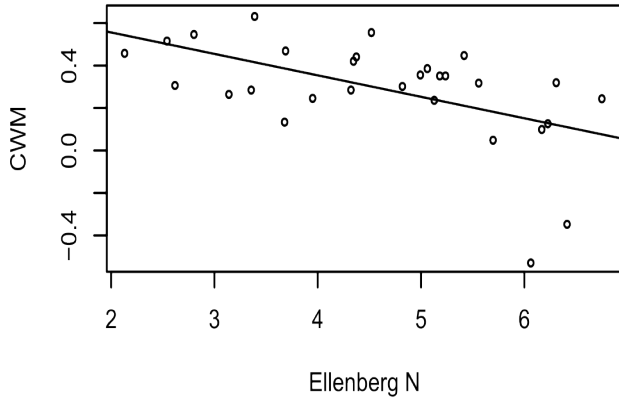
Height



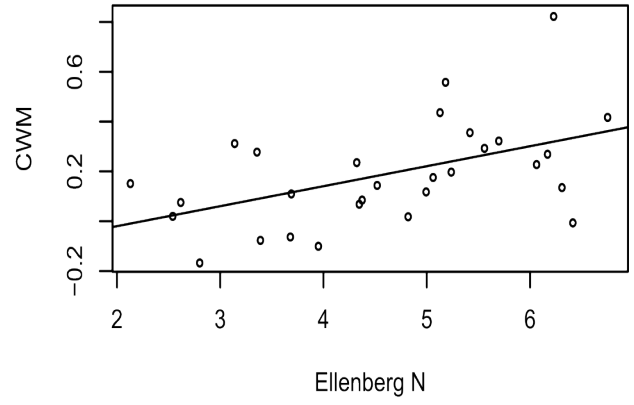
Bud bank size



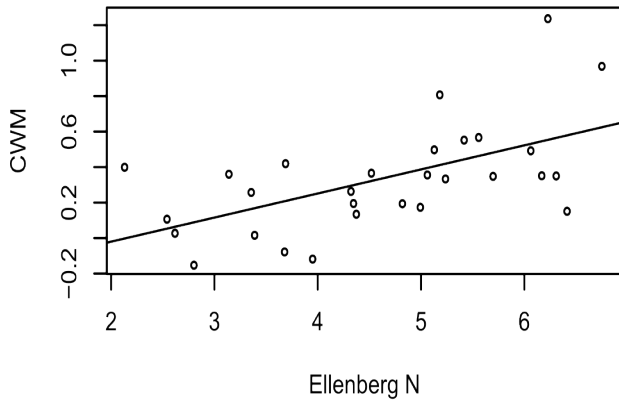
Bud bank size including root taps



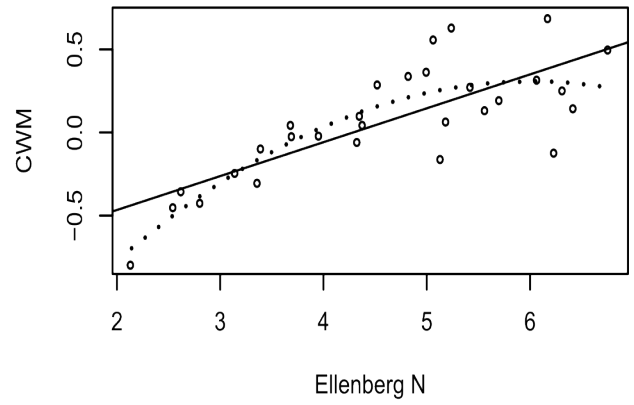
All bud bank traits



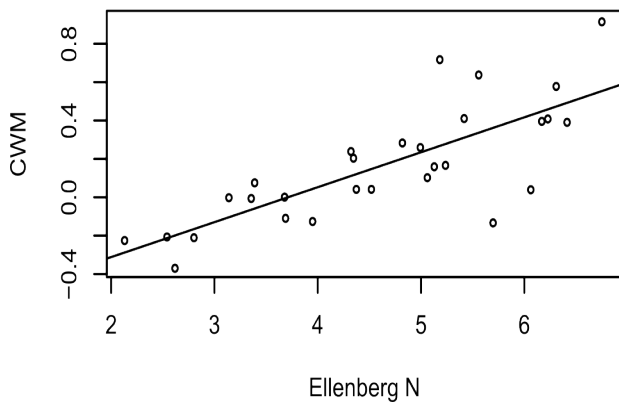
Multiplication rate



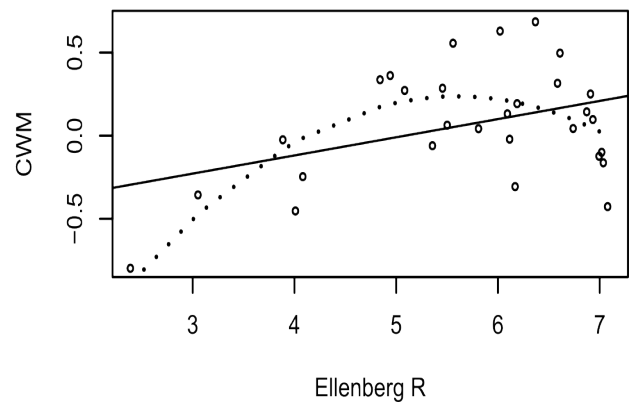
All Clonal traits



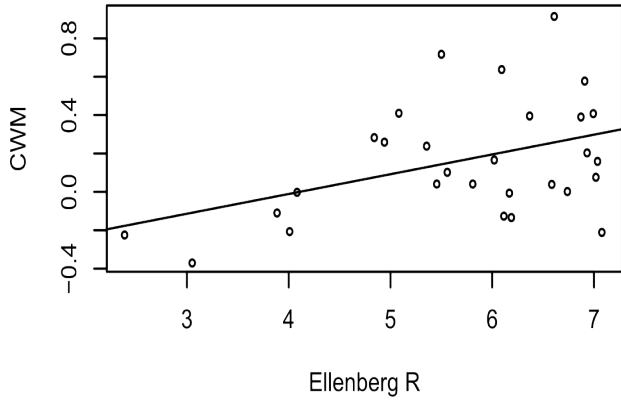
Clonal and bud bank traits



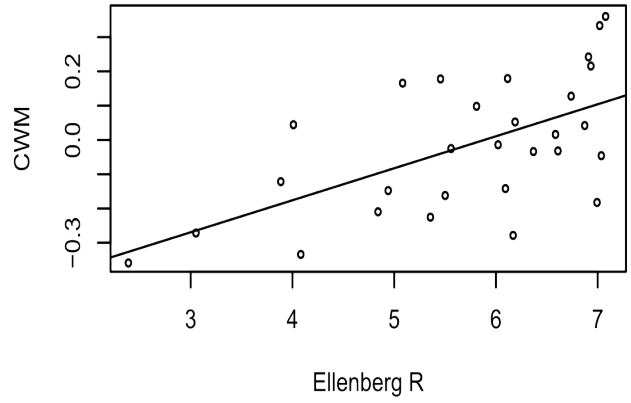
Specific leaf area



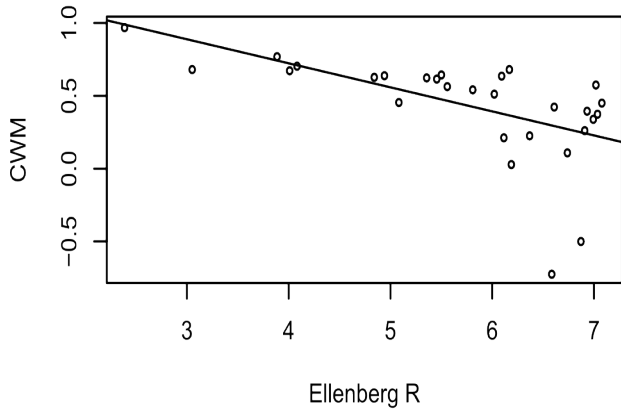
Height



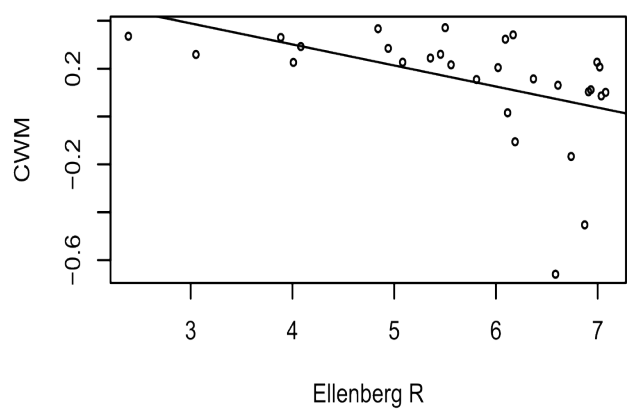
Root sprouting capacity



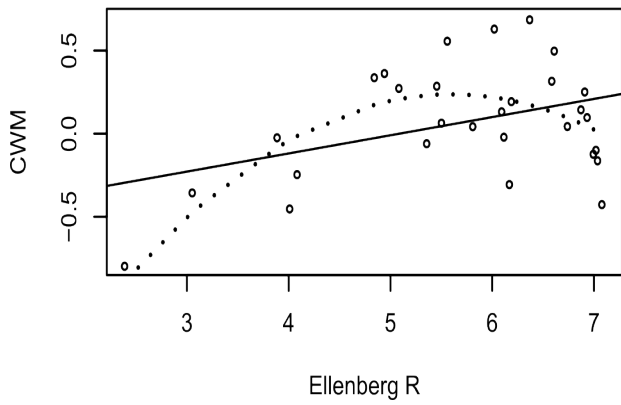
Bud bank size



Bud bank depth



All Clonal traits



Clonal and bud bank traits

