UNIVERSITÀ DEGLI STUDI DI MILANO

Biosciences Department

Ph.D. course in Environmental Sciences

XXXIII cycle

Trait-based functional characterization of plant species and communities: trends and adaptions to environment in Alpine and European vegetation

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Academic year 2019/2020

This Ph.D. project was funded by FLA – Fondazione Lombardia per l’Ambiente, via University of Insubria (Varese), on account of the Regional Observatory for Biodiversity. Lab measurements and analyses were carried out with the Unit of Vegetation Ecology and Phytogeography of the Biotechnology and Life Sciences Department (DBSV) of the University of Insubria (Varese), under the supervision of prof. Bruno E.L. Cerabolini.

CONTENT

Introduction

1. Functional approach to plant ecology

Since the ancient times, the description and the classification of organisms has always been based on their structure and appearance. Plants, in particular, have often been categorized on the basis of their morphology and physiognomy; in fact, until the 19th century, physiognomic types were the primary descriptive units of a plant community and of the vegetation of a specific region (Du Rietz 1931). Later on, during the early 20th century, some attempts were made to arrange plants into groups that emphasize the ecological significance of plant forms (e.g. Warming 1895, 1909; Schimper 1903), until the well-known and widely applied life-forms classification system of Raunkiær (1934), which categorizes plants by the position of their perennating organs during the most unfavourable season.

Simultaneously, advances in evolutionary studies and genetics allowed to propose another classification system that takes into account the degree of relatedness between organisms; thus, plants (and animals) were situated in systematic categories based on their evolutionary history and their phylogenesis (Garnier et al. 2016).

Only in the 1980s ecologists began to consider that plants diversity transcends form and taxonomy, and that particularly relevant is the function that each organism carries out in relation to the environment it occupies (Garnier et al. 2016). This functional approach allowed the definition of functionally homogeneous groups of plants (i.e. functional types) which are ‘sets of species showing similar responses to the environment and similar effects on ecosystem functioning’ (Smith et al. 1992; Díaz and Cabido 1997, 2001; Lavorel and Garnier 2002). Species, therefore, can be classified into groups that relate directly to function through shared characteristics, rather than phylogeny (Lavorel et al. 1997); such characteristics are called plant functional traits.

2. Plant functional traits

Plant functional traits are morphological, physiological or phenological features, measurable at the individual level, with a direct or indirect effect on whole-plant fitness (McGill et al. 2006; Violle et al. 2007; Gillison et al. 2013). They are plant characteristics readily measurable and related to its functioning (Fig. 1), that have been widely used in functional ecology over the last few decades to better understand the interactions between plants and the abiotic and biotic components of their environment. Indeed, a trait-based approach offers many insights into general responses to ecological factors, plant resource acquisition and use, plant assemblages and relations within and between communities, from individual to ecosystem scale (Garnier and Navas 2012; Reich 2014). In fact, plant traits can influence the functioning and the dynamics of entire ecosystems since they play an important role in affecting and regulating ecosystem processes and services provision (Díaz and Cabido 2011; Garnier et al. 2016).

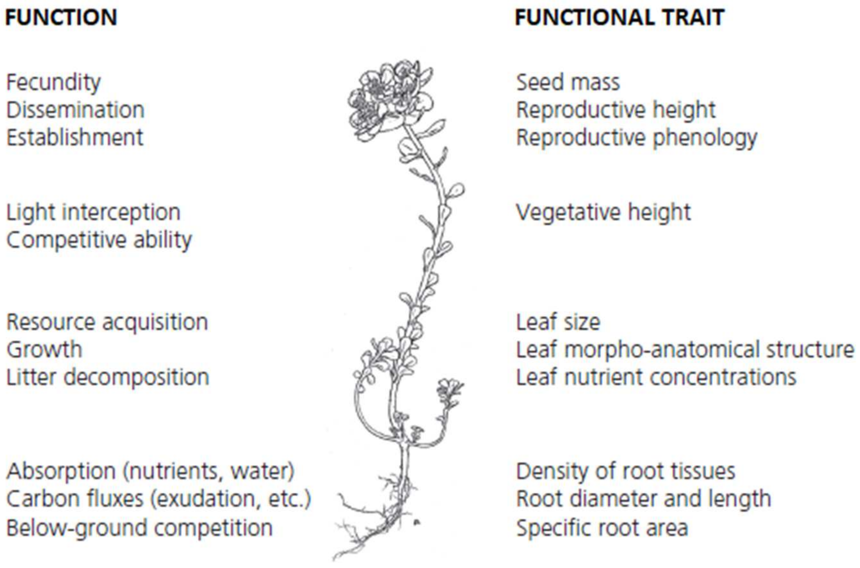


Figure 1: Examples of functions defined at the individual level and corresponding functional traits linked with them (modified from Grarnier et al. 2016).

It is recognized that plant trait variation shows several trade-offs and correlations (see the following paragraphs for further details) which means that a small number of traits carries most information about vegetation adaption to a broad range of environmental aspects and, overall, about ecosystem functioning. These core traits are directly related to fundamental plant functions that are common to all species: growth (i.e. establishment), survival (i.e. persistence to a range of abiotic and biotic conditions) and reproduction (i.e. diaspore production and dispersion) (Weiher et al. 1999).

2.1 Global spectrum of plant form and function

Díaz et al. (2016) demonstrated at a global scale that the functional space occupied by vascular species is strongly constrained by trade-offs between combination of six key traits (adult plant height, stem density, leaf area, leaf mass per area, leaf nitrogen content and seed mass). They demonstrated that these traits do no vary independently and that their variation converged toward a particular pattern, identifying the global spectrum of plant form and function (Fig. 2). Such pattern consists of two main axes of variation: one represents trade-offs between the ability to capture light, disperse diaspores and growth potential, while the other represents the trade-offs between leaf resource acquisition and conservation. Precisely, the first axis describes the size variation of the plant and its organs, while the second axis corresponds to the leaf economics spectrum (LES) (Wright et al. 2004; Reich et al. 2014). The LES reflects leaf traits covariation between species characterized by short-lived leaves, fast growth and rapid nutrient acquisition, and species with long-lived leaves with a main tendency toward resource conservation; it describes the productivity-persistence trade-off that is noticeable in all vascular plant species. This same pattern in plant trait variation is also found at the community level as revealed in a recent global scale study (Bruelheide et al. 2018), which demonstrated that the ecological drivers that shaped species-level trait trade-offs are also reflected in the composition of plant communities.

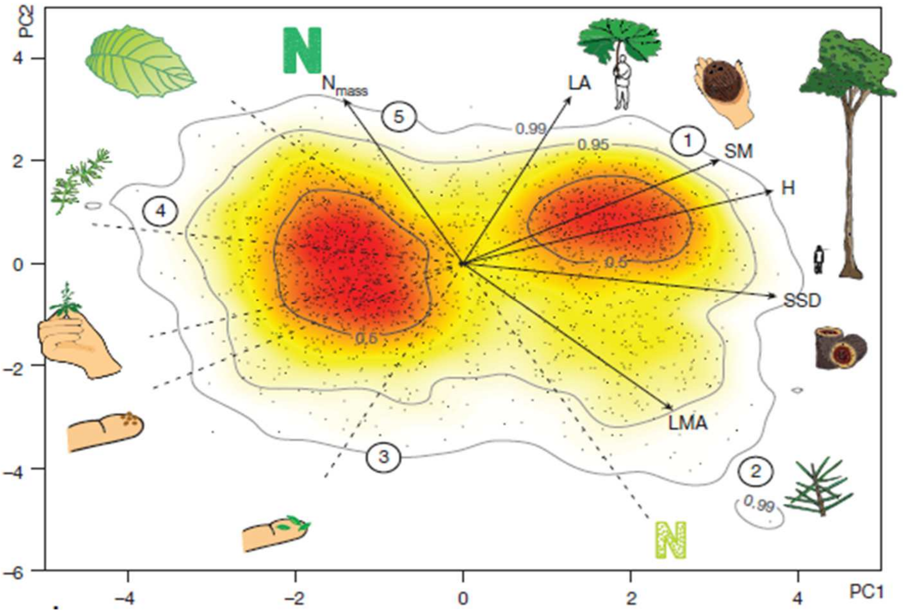


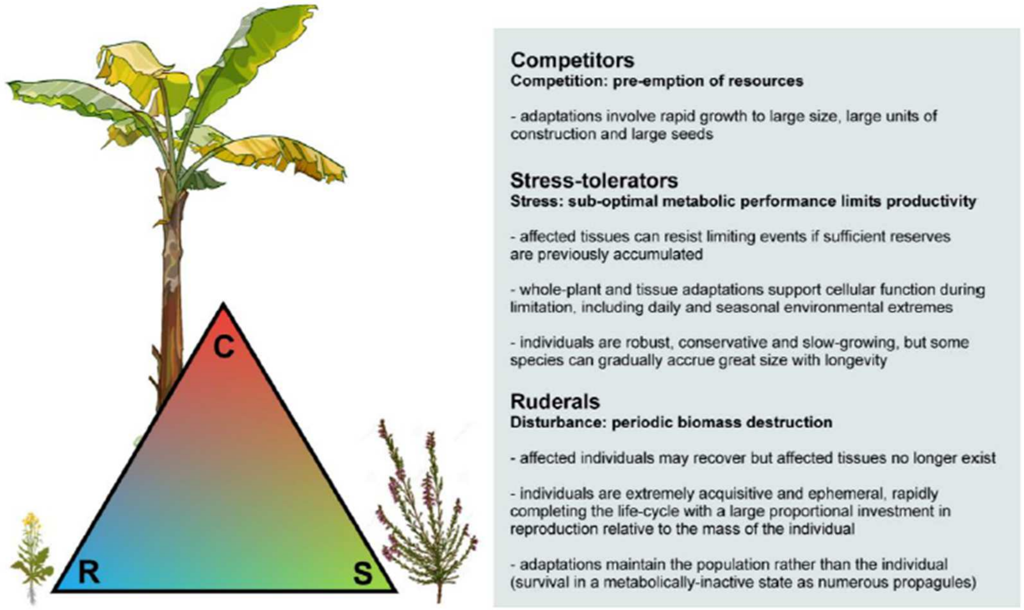
Figure 2: The two main axes of variation of vascular plant species identified by Díaz et al. (2016): one going from short plants with small organs (lower left corner) to tall plant with big organs (upper right corner), and the other going from species with large and acquisitive leaves (upper left corner) to species with small and conservative leaves (lower right corner). The colour gradient indicates regions of highest (red) to lowest (white) occurrence probability of species in the trait space. The left region corresponds to herbaceous species, while the right region corresponds to woody species (from Díaz et al. 2016).

2.2 CSR plant adaptive strategies

As mentioned above, it is widely acknowledged that plant responses to ecological factors involve combinations of a small number of traits that co-vary recurrently among environments, identifying certain axes of variation characteristic of the different strategies of plant functioning (Garnier et al. 2016). Thus, plant strategies reflect viable suites of characteristics that best maximize the overall plant fitness and survival in a specific habitat (Pierce et al. 2017); in fact, particular trait values are systematically found in similar environmental contexts, even though the taxonomic composition differs (Shipley 2010).

Central to the development of a strategy model is the identification of the principal selective forces that shaped plant traits and resulting strategies. Grime (1974; 1979; 2006) proposed a three-way strategy model, one of the most prominent and recognized, in which distinct trait combinations are selected under conditions of competition, abiotic limitation to growth (i.e. stress) or periodic biomass destruction (i.e. disturbance), respectively. Specifically, plants are classified as competitors (C) when they live in stable and productive habitats, with efficient resource acquisition, large size and big organs; alternatively, they are classified as stress-tolerators (S) when are adapted to survive in variable and resource-poor environments by investing in resources conservation with dense and persistent tissues. Finally, plants are considered ruderal (R) when are adapted to endure disturbance and repeated biomass destruction events through rapid growth and major resource investment in propagules to ensure regeneration (Pierce et al. 2017) (Fig. 3). Grime (1979) also proposed 'secondary' strategies for species adapted to habitats with moderate intensities of stress and disturbance and intermediate resource availability: competitive ruderal (C-R), stress-tolerant ruderal (S-R) and stress-tolerant competitor (S-C).

Pierce et al. (2017) developed an extensively recognized and applied method to assign all vascular plant species to a certain strategy based on three leaf traits: specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area (LA). These traits are widely available for a large number of species and represent the size (i.e. LA) and the opposite extremes of the leaf economics (i.e. SLA and LDMC) spectra which are major components of the CSR strategy model (Pierce et al. 2012). Indeed, variation in leaf size and economics traits is a global phenomenon evident in a variety of lifeforms and highly representative of functional trade-offs. Moreover, these traits are associated with whole-plant and reproductive trade-offs so that it is assumed that their variation is representative of a large portion of overall plant variation (Díaz et al. 2016; Pierce et al. 2017).



Competitors
Competition: pre-emption of resources

- adaptations involve rapid growth to large size, large units of construction and large seeds

Stress-tolerators
Stress: sub-optimal metabolic performance limits productivity

- affected tissues can resist limiting events if sufficient reserves are previously accumulated
- whole-plant and tissue adaptations support cellular function during limitation, including daily and seasonal environmental extremes
- individuals are robust, conservative and slow-growing, but some species can gradually accrue great size with longevity

Ruderals
Disturbance: periodic biomass destruction

- affected individuals may recover but affected tissues no longer exist
- individuals are extremely acquisitive and ephemeral, rapidly completing the life-cycle with a large proportional investment in reproduction relative to the mass of the individual
- adaptations maintain the population rather than the individual (survival in a metabolically-inactive state as numerous propagules)

Figure 3: Schematic representation of Grime's CSR plant strategy theory with definitions of competition, stress and disturbance, and the relative adaptive plant responses (from Pierce and Cerabolini 2018).

Among key leaf traits, those related to leaf nutrient content are also highly relevant to describe plant variation in resource acquisition and conservation. Particularly, nitrogen, phosphorus and carbon have received much attention due to their large contribution to plant growth, and nitrogen, specifically, has been identified as the main representative of global scale trade-offs between leaf economics (Díaz et al. 2016); high levels of leaf nitrogen content (LNC) are associated with rapid resource intake while the carbon to nitrogen ratio (C/N) reflects a predominance of resource conservation (Pierce et al. 2007a; Freschet et al. 2010; De La Riva et al. 2018). Other elements have been less investigated, probably because of their more limited relevance to plant productivity (Mitchell et al. 1992); however, sulfur is one of the macronutrients essential for plant metabolism and physiological functions, such as photosynthesis and the composition of organic compounds (e.g. proteins, vitamins and antioxidants) (Marschner 2012; Capaldi et al. 2015). Leaf sulfur content (LSC), therefore, could be another important trait to be considered in leaf economics trade-offs and plant strategies; this point is discussed in Chapter 2 where this leaf trait is analyzed within the LES and the CSR adaptive strategies frameworks, to explore its variation alongside other major axes of plant adaption and to assess whether it could be recognized as an addition to the acknowledged core trait set.

3. Trait - environment relations

Differences between the values of plant traits among species can be partially explained by their evolutionary history and their phylogeny; a relevant part of this variability though, is caused by the responses of plants to contrasting environmental conditions which selected the traits and the trait values that allowed plants success (i.e. establishment, persistence and reproduction) in those habitats. Environmental factors influence plant functions by acting as filters that identify, from a regionally available pool, which trait combinations are successful and which are not; plant communities can be considered as the result of environmental abiotic and biotic processes that constrain which species bearing certain traits can successfully persist at a site (Lavorel and Garnier 2002; Violle et al. 2007; Díaz et al. 2016). In other terms, variation in plant functioning (captured by variation in trait values) and species distribution depend on variation in environmental factors and have local adaptive value (Garnier et al. 2016).

Such factors can change gradually through space and time identifying gradients, a concept introduced by Whittaker (1951; 1967), central to ecology, which can be determined by different variables such as altitude, latitude, successional stage, resource availability, soil composition, disturbance events (grazing, fire, trampling, floods etc.) and many others. Accordingly, plant functional traits values have been shown to change along ecological gradients (e.g. Ackerly et al. 2002; Shipley et al. 2017), but the majority of these studies analysed trait variation in response to general gradients that included different factors, such as climate, stress, and disturbance (Díaz et al. 1998; Schöb et al. 2013; Herben et al. 2018). The link between trait variation and gradients of a single ecological driver has rarely been systematically evaluated, although it could be highly relevant to assess and predict current and future trait values distribution, also within the global change context (Butler et al. 2017; Wright et al. 2017). In Chapter 3 is proposed the application of Environmental Indicators (Ellenberg et al. 1992; Landolt et al. 2010) as representative of each species niche within the range of key ecological drivers (temperature, continentality, light, soil moisture, soil pH and soil nutrients), to examine inter-specific trait variation along Ecological Indicators gradients and, thus, plants functional responses to changes in single ecological factors.

As mentioned above, plants success in a given environment depends on their response to a range of biological and physical factors, embodied by their functional trait values. In particular, suites of traits can more effectively explain viable plant characteristics that positively affect each species fitness; CSR adaptive strategies theory summarizes multiple traits that are involved in plant resource economics and size, and can thus be useful to assess the success (in terms of establishment, persistence and reproduction) of different species in certain habitats (Pierce et al. 2017).

Among plant species, it is especially important to evaluate the success of invasive alien species because of their known ability to rapidly expand into existing native communities, causing major negative impacts on biodiversity, ecosystem functioning and services (Richardson et al. 2000; Pejchar and Mooney 2009; Vila et al. 2011). The spreading of these alien species is constantly increasing and is also severely damaging human well-being under economic and social aspects (Pyšek et al. 2017). Therefore, it is important to identify the characteristics that allow these species to invade plant communities and to outcompete native species in different environments, in particular to develop effective containment measures. This issue was addressed in Chapter 4 where CSR plant adaptive strategies are applied to assess differences and similarities between the adaptive trade-offs of native and invasive alien species, and to determine the prevalent strategy of the latter that determine their success and which environments are more exposed to the threat of alien invasion.

4. Functional diversity

Biodiversity is a very broad concept that encompasses the variability of every aspects of organisms, from genes to ecosystems level. Although it is often associated with taxonomic diversity alone (i.e. the number of species at a given site), differences among living beings present many other facets (e.g. phylogenesis, organisms interaction, structure, plant assemblages etc.). Particularly relevant are the different functions that each individual (or species) carries out in a community; in fact, species largely differ ecologically and play different roles or functions in ecosystems (Shulze et al. 2019). This aspect of biodiversity is the functional diversity, which has been defined by Tilman (2001) as ‘the range of values of those organism traits that influence the functioning of an ecosystem’; it is of ecological importance because, by definition, is the biodiversity component that affects all aspects of ecosystem properties and processes, including the delivery of ecosystem services (Petchey and Gaston 2002; Garnier et al. 2016), and has a great potential for exploring the effects of community structure on ecosystem functioning (Hooper et al. 2005; Grime 2006). Indeed, as indicated by Ricotta and Moretti (2010), several studies concluded that functional diversity is highly correlated with ecosystems functions as productivity (Tilman et al. 1997; Hooper and Dukes 2004; Petchey et al. 2004; Hooper et al. 2005), resilience to perturbations or invasion (Dukes 2001; Bellwood et al. 2004), and regulation in the flux of matter (Waldbusser et al. 2004).

Nevertheless, there is no unique, simple or standardized method to determine functional diversity, since many plant characteristics have to be evaluated (Díaz and Cabido 2001; Tilman 2001; Petchey and Gaston 2002). In fact, Mason et al. (2005) and Villeger et al. (2008) argued that functional diversity cannot be adequately summarized by a single measure, but they assert that it includes three separate components: (1) the amount of functional trait space filled by species in the community (functional richness); (2) the evenness of abundance distribution in the filled trait space (functional evenness); and (3) the degree to which the distribution of species abundances maximizes divergence in functional traits (functional divergence). Nonetheless, a variety of measures have been proposed to summarize the range and distribution of trait values in a given plant community; which one to apply depends on the available information and the aims of the research (Pla et al. 2012). For instance, one option is the estimation of the number of functional groups represented by the species in a community (Fonseca and Ganade 2001; Tilman 2001), another is the analysis of the functional distances between species pairs in multivariate trait space (Botta-Dukat 2005; Ricotta 2005; Schmera et al. 2009) or of the distances between species along hierarchical and non-hierarchical classifications (Petchey and Gaston 2002; Ricotta and Moretti 2008). Otherwise, functional diversity may be summarized using functional diversity indices that are based on trait values measured at species level; they may also incorporate measure of the species abundance in the community. For a detailed review of functional diversity indices see Pla et al. (2012).

According to Grime (1998), the extent to which a plant species influence ecosystem functions is closely related to its contribution to the total plant biomass of the community. This is the mass ratio hypothesis which concludes that, even in species-rich environments, most of the plant biomass may reside in a small number of dominant species, the characteristics of which are likely to override as ecosystem controllers the effects of more numerous subordinate or transient species. Consequently, ecosystem properties should be largely determined by the functional diversity among the dominant species rather than by the number of functional groups present in the community. A metric usually applied to express the functional composition of a plant community is the Community Weighted Mean, CWM (Garnier et al. 2004; Lepš et al. 2011), the mean trait value of each species weighted by the abundance of that species in the community, that embodies the extent of a trait presence in a plant assemblage (Pla et al. 2012).

In Chapter 5 this metric has been applied to provide a detailed assessment of ecosystem functioning, through the analysis of trait-environment interactions and CSR adaptive strategies, in plant communities of a pasture in the Italian central Alps. This chapter, thus, deals with functional diversity at the community level (i.e. between communities) examining multiple abiotic and biotic variables (especially soil properties) changes following a topographic sequence along a mountain slope characterized by high soil and vegetation variability at a detailed scale, to provide further insights into the capability of functional diversity to reflect underlying ecological processes.

Patterns of traits and Grime's CSR strategies variation at the community level, especially along gradients of vegetation succession at a regional scale, are still little explored as most of the studies on this subject are conducted at the local scale (e.g. Caccianiga et al. 2006; Pierce et al. 2007b; Ricotta et al. 2015). In Chapter 6 this issue is addressed by analysing the variation of CWMs of key traits (LA, SLA, LDMC) and CSR adaptive strategies of six floristically and ecologically defined alpine plant communities, corresponding to three Habitat types of EU Community interest (Habitats Directive, 92/43/EEC). These communities were also representative of a successional gradient in alpine environment on siliceous substrate, going from scree and moraine vegetation to grasslands and shrubs, which allowed the identification of realized functional niches and a general functional interpretation of this habitat.

5. Functional ecology and phytosociology

Plant classification according to functional ecology, as stated earlier, is based on their adaptive traits and on the identification of functionally homogeneous groups (i.e. functional types), reflecting species assemblages responding coherently to abiotic and biotic environmental factors. The widely applied and well-known traditional phytosociological classification system, proposed by Braun-Blanquet in the 1920s, instead, categorizes vegetation according to the occurrence or abundance of species recorded in plots (Chytrý and Thichý 2003; De Caceres et al. 2015). In particular, phytosociology presumes the definition of categories, called *syntaxa*, included within a floristically defined hierarchical classification system; the identification of each *syntaxon* (for each rank level) is based on the presence of common groups of diagnostic species (for definition see Chytrý et al. 2002).

However, although relations between vegetation communities and environmental factors are considered by both functional ecology and phytosociology (as *syntaxa* are defined in relation to a given ecological context), a connection between these two approaches to plant classification and description is yet to be assessed. This issue was addressed in Chapter 7 by the functional analysis, through plant traits and CSR strategies, of woody diagnostic species of plant communities defined by phytosociology. In details, the study was at the class level, which is the most comprehensive rank, across a broad range of different habitats of European woody vegetation, from low shrub to forest formations and including form mediterranean to boreal/alpine climatic belts.

For details on traits measurement, datasets and study area see Appendix 1 and 2.

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Project aims

The main aim of this project was the functional characterization, through the application of plant functional traits and Grime's CSR adaptive plant strategies, of plant communities of European vegetation at different levels (within and between communities), in order to highlight functional similarities and/or differences and to identify common patterns and responses to environmental factors.

The punctual goals were:

- explore the role of other traits related to leaf nutrient content in the framework of the leaf economics spectrum and CSR strategies, to be considered as an addition to the acknowledged and commonly used core trait set; in particular, it was examined leaf sulfur content (Chapter 2);
- assess plant inter-specific trait variation in response to changes in single ecological factors, by considering gradients of different Ecological Indicators referring to key environmental drivers (Chapter 3);
- define how functional traits can affect plant success and adaption to different habitats, also in the context of global change, by comparing CSR strategies of alien invasive species and native species (Chapter 4);
- analyse functional variation between multiple plant communities along a topographic sequence in relation to a variety of abiotic and biotic factors, by considering CSR strategies of different plant communities in an alpine pasture (Chapter 5);
- functionally characterize, through trait variation and CSR strategies, multiple plant communities along a successional gradient at a regional scale; in particular, were considered floristically and ecologically defined communities corresponding to Habitat of EU Commmunity interest (Chapter 6);
- identify a link between the two main approaches to vegetation description and classification, phytosociology and functional ecology, by the functional characterization of classes of European vegetation using their woody diagnostic species (Chapter 7).

The contribution of leaf sulfur content to the leaf economics spectrum explained by plant adaptive strategies

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Submitted to *Environmental and Experimental Botany* on 02-11-2020

Abstract

Sulfur is an essential macronutrient for plant primary metabolism and growth, but may be toxic when in excess. Terrestrial ecosystems have faced extensive anthropogenic sulfur depositions during the 20th century, but currently decreasing trend of sulfur emissions suggest that it could become limiting, although its relationship with leaf functional traits remains unclear.

We analysed leaf and nutrient traits for 740 vascular plant species growing in a wide range of environmental conditions in Northern Italy. We aimed to determine whether leaf sulfur content (LSC) is associated with the leaf economics spectrum, and whether its distribution among functional types (life forms, leaf life span categories, and Grime's CSR (Competitive, Stress-tolerant, Ruderal) strategies) could help to elucidate adaptive differences within plant taxa.

High LSC values were associated with R-strategy selection, hence the acquisitive extreme of plant economics, reflecting strong potential interactions with ecosystem properties such as biomass production or litter decomposability. We also found evidence of a relationship between nutrient stoichiometry and taxonomy, specifically at the acquisitive and conservative extremes of leaf economics. In general, LSC was significantly and positively correlated with leaf nitrogen content, but ruderal strategies in particular exhibited greater sulfur to nitrogen ratios.

Our findings highlight that, faced with a dearth of LSC data, leaf nitrogen content can be used as a coarse proxy of LSC within the context of plant economics. However, different ratios among sulfur and nitrogen may be expected for ruderal species, suggesting that deeper insight from CSR strategies can provide a bridge between plant stoichiometry and ecology, useful for the evaluation of ecological responses to global change.

Key words: Plant functional traits, Grime's strategies, CSR, leaf nutrients, ecological stoichiometry

1. Introduction

Sulfur is one of the macronutrients essential for plant growth and physiological functions and is vital for metabolic processes (Aerts and Chapin 1999, Marschner 2012). Sulfur availability has a particularly important effect on photosynthesis because it is a component of the proteins involved in chlorophyll synthesis (Hu et al. 2017) and chloroplasts formation (Hasanuzzaman et al. 2018). Hence, sulfur limitation decreases yields and quality parameters of crops (e.g. Hawkesford and De Kok 2007, Bouranis et al. 2008) and the growth of tree species (e.g. poplar; Honsel et al. 2012). Demand for sulfur varies strongly between species (Buchner et al. 2004), but its uptake and assimilation are highly dependent on the availability of nutrients and on environmental conditions (Davidian and Kopriva 2010). Sulfur assimilation is linked to multiple metabolic pathways responsible for a range of physiological functions (Capaldi et al. 2015), mainly including primary metabolite biosynthesis, stress responses, and pathogen defence. Aside from effects on productivity, sulfur deficiency may thus also result in the loss of plant resistance to environmental stress and pests.

Together with nitrogen, sulfur is a component of essential organic compounds, such as proteins (amino acids cysteine and methionine), vitamins (biotin and thiamine), cofactors (Co-A and S-adenosyl methionine) and various other secondary compounds (Marschner 2012). Indeed, protein conformation (secondary and tertiary structure) depends on disulfide bridges between amino acid components, and sulfur is thus a fundamental requirement of living systems. Moreover, sulfur protects plants from biotic (i.e. herbivory; Badanes-Perez et al. 2014) and abiotic stresses such as salinity, drought, and toxic metals/metalloids (Na and Salt 2011, Urbina et al. 2015, Hasanuzzaman et al. 2018), through different compounds that directly act as antioxidants or modulate antioxidant defence systems (e.g. glutathione, phytochelatins, metallothioneins) (Hawkesford and De Kok 2007). In addition, plants may emit minute amounts of volatile sulfur compounds, such as H_2S, which may protect plants, for example against fungal attack (Hasanuzzaman et al. 2018).

Under natural conditions, the major sulfur source for plants are soil sulfates that originate from the mineralization of organic matter, which is sometimes a limiting step for plant sulfur supply, particularly in agricultural systems with low sulfur fertilisation (Eriksen 1997). However, plants may acquire, through the leaves, other sources and forms of sulfur for growth, e.g. smaller quantities of sulfur dioxide (SO_2; De Kok et al. 2017) coming from deposition following either natural (volcanic activity or sulfur springs) or anthropogenic (industrial) emissions (Bussotti et al. 2005). Indeed, until the 1980s, the amount of sulfur fertilization tended to increase along with SO_2 emissions due to industrial activity, but simultaneously SO_2 was the main contributor of the significant intensification of acid deposition such that sulfur was mainly regarded as a pollutant causing forest die back (e.g. Schulze et al. 1989). Following the steady increase during the beginning of the 20th century, national and international legislation on emission-reductions lead to the current worldwide trend of decreasing SO_2 emissions (Aas et al. 2019), and to a sulfur deficiency scenario negatively affecting yield and quality of agricultural crops and biomass production of natural ecosystems. The extent to which sulfur could become a limiting element for both crops and wild species is unknown (Johnson et al. 2018). In order to understand and predict the effects of sulfur limitation, the resource economics of this nutrient (i.e. how plants manage and allocate mineral elements between different functions) must be determined. In other words, different species potentially exhibit different sulfur use in metabolism and concomitantly vary in growth responses, depending on life form and ecological strategy.

Despite the relevance of sulfur for plant metabolism, much attention has been paid to carbon, nitrogen and phosphorous, due to their larger contribution to plant growth, while few studies have investigated the stoichiometry of sulfur in terrestrial ecosystems (Han et al. 2011, Legay et al. 2014, Miatto and Batalha 2016, Shi et al. 2016, Wu et al. 2017), probably because it is a less critical limiting factor for plant productivity (Mitchell et al. 1992). Leaf nutrient contents of terrestrial plants are typically related to environmental factors (see Dalle Fratte et al. 2019a), but sulfur in particular can also be related to taxonomy (Zhang et al. 2012, Wu et al. 2017, De La Riva et al. 2018). For example, glucosinolates are sulfur-containing plant resistance compounds found mainly in species of the *Brassicaceae* family (Dijkshoorn and Van Wijk 1997), but have been recorded from 16 families in two main 'mustard oil' clades (Rodman et al. 1998). Among leaf nutrient contents, nitrogen has been identified as the main representative of global scale trade-offs between resource capture and conservation (Díaz et al. 2016). Such variation in plant resources allocation has been extensively investigated within the context of the leaf economics spectrum (LES; Wright et al. 2004, Reich 2014). Rapid resource acquisition is usually correlated with high values of specific leaf area (SLA) or leaf nitrogen content (LNC), while high leaf dry matter content (LDMC), lignin content or carbon to nitrogen ratio (C/N) reflect a resource conservation strategy (Pierce et al. 2007, Freschet et al. 2010, De La Riva et al. 2018). Since the larger portion of sulfur is used for primary metabolite biosynthesis, it is expected that leaf sulfur content (LSC) as well as the carbon to sulfur ratio (C/S) are embroiled in the LES.

The interactions of LSC with other plant traits has been studied only locally and/or for a few species (e.g. Bussotti et al. 2005, Sardans et al. 2008, Laliberté et al. 2012, Urbina et al. 2015). De La Riva et al. (2018) highlighted that the use of functional groups (leaf life span and habitat type) appears to be useful to untangle the relationship between LSC and plant functioning. Grime's CSR (Competitive, Stress-tolerant, Ruderal) adaptive strategies scheme (Grime 2006) defines functional groups identified on a solid theoretical base, and is the only ecological strategy theory that simultaneously explains both economics and size as fundamental gradients of plant adaptation and evolution (Grime and Pierce 2012). Considering that the extremes of the LES roughly correspond to stress-tolerant (conservative) and ruderal (acquisitive) CSR strategies (Díaz et al. 2016), these should also be connected to LSC. Despite this, we are not aware of comparative studies of LSC in relation to CSR strategies.

Here, based on a large dataset of the vascular flora of Northern Italy, representative of a wide range of environmental conditions, we tested whether: 1) LSC varies in concert with major axes of plant adaptation, in particular with the LES and consequently, 2) LSC distribution is related to specific life-forms, leaf life span categories and plant families along the LES, 3) LSC relates in different ways to CSR adaptive strategies so that these can help to explore LSC within taxonomic or functional groups.

2. Methods

2.1 Dataset

Our dataset (LIFTH, Leaf and nutrient Italian Flora Traits Hoard) consists of 740 species belonging to 99 families, of which only 17 are represented by at least 10 species records (Table A1). The dataset includes leaf and nutrient traits of both wild species characteristic of the main habitats of Northern Italy, and domesticated species (i.e. only cultivated in gardens and public parks). Nomenclature of every taxon (family, genus, species) in our dataset was standardized according to The Plant List (TPL, www.theplantlist.org) using the R package 'Taxonstand' (Cayuela et al. 2017).

For each species, within the same population we sampled from 5 to 15 fully expanded leaves selected randomly from the outer canopy of different individual adult plants growing in nature. Sampling sites were widespread over an area of approximately 50,000 km² ranging between 43°16' – 46°34' N latitudes and 07°54' – 11°00' E longitudes, with an altitudinal range of 2760 meters (30 - 2790 meters a.s.l.), i.e. from sea coasts to higher mountain belts. Indeed, even though most of the species in our dataset are representative of vegetation types of the Alpine (Southern Alps) and the Continental (Po Plain) Biogeographical Regions (ETC/BD 2006), an appreciable number of samples (7.3 %) were collected within the Mediterranean region. Consequently, climate regimes, geological substrates and soils of the sampling sites show extensive variability, even at the local scale (Smiraglia et al. 2013, Pesaresi et al. 2017).

Laboratory measurements followed a standardized methodological protocol (Perez-Harguindeguy et al. 2016): we stored leaf material at 4 °C overnight to obtain full turgidity for the determination of leaf fresh weight (LFW) and leaf area (LA; i.e. the surface area of fully expanded leaves); petioles and rachides were included as part of the leaf. LA was determined using a digital scanner and the software Leaf Area Measurement (LAM v.1.3; University of Sheffield, UK). Leaf dry weight (LDW) was then determined after drying for 24 h at 105 °C, and parameters such as SLA (ratio between LA and LDW) and LDMC (ratio between LDW and LFW) were calculated. Hence, for each species we computed the mean value of all plant traits. We also derived CANH (CANopy Height) from literature data (Pignatti 1982). The dry leaf material was then mixed and pounded, and three randomly selected replicates were processed with a CHNS-analyzer (FlashEA 1112 series Thermo-Scientific), obtaining values of LCC (Leaf Carbon Content), LHC (Leaf Hydrogen Content), LNC (Leaf Nitrogen Content) and LSC. For each of the three replicates, we also calculated the ratio between LCC and LNC as well as LSC (respectively C/N and C/S), and averaged values for each species. Finally, using LA, SLA and LDMC, we calculated the C-, S-, and R- scores, according to the *StrateFy* tool of Pierce et al. (2017) and then classified species into seven CSR strategy categories: C, CR, CSR, R, SR, S, SC (Grime, 2006). The *StrateFy* tool (Pierce et al. 2017) compares the trade-offs between LA, SLA and LDMC for each target species against worldwide variation in these traits, allowing the extent of acquisitive vs. conservative leaf economics and plant size to be quantified and compared against absolute global limits. This method mirrors variability in fourteen whole-plant, flowering, seed and leaf functional traits (Pierce et al. 2017) and has the advantage of using trait variation evident amongst vascular plants in general, including woody and herbaceous species and taxa such as ferns, and can thus be applied to large datasets of wild plant species and their communities.

We performed the analyses using nine plant traits: LA, LDW, SLA, LDMC, CANH, LNC, and C/N plus sulfur traits (LSC and C/S). We selected these traits because they represent the major axes of plant adaptation worldwide (Díaz et al. 2016) and/or are used for the computation of CSR scores (Pierce et al. 2017). We added LDW to account for the accumulation of dry matter of leaves aside from LA (Pierce et al. 2013), while C/S was included because it represents (along with C/N) a trait related to litter decomposition and carbon balance (Blair 1988, Freschet et al. 2010, Pierce et al. 2007).

We categorised species into four life form categories following Dalle Fratte et al. (2019b): trees (n = 86), shrubs (n = 118), long-lived herbs (n = 433), and short-lived herbs (n = 103). Such broad categories largely correspond to those of Raunkiær (1934) and help to remove growth forms composed of few species (e.g. lianas, herbaceous vines, rushes). Essentially, this classification divides woody species (n = 204) into trees and shrubs (shrubs and lianas), and non-woody species (n = 536) on the base of their life cycle length, regardless of being graminoids, forbs, aquatics or herbaceous vines: short-lived (which include annual and biennal) vs. long-lived (perennial) herbs.

We also classified species according to their leaf life span, which plays a critical role in plant economics (Reich 2014). We adopted three categories summarising the attribute ‘leaf duration’ proposed by Landolt et al. (2010) for the Swiss flora: evergreen (n = 64), wintergreen (n = 308), deciduous (n = 368). Accordingly, evergreen are those plants having leaves or needles all year round and older than one year, while deciduous have leaves that die off after having been green during summer, and only rarely some leaves may remain green during mild winters. Wintergreen species are those plants that build leaves during the vegetation period, but they remain green until the next leaf unfolding in spring unless they are exposed to very low temperatures or extreme drought.

2.2 Statistical analysis

We computed all the statistical analyses using the R software (R Core Team 2020). Data were first normality checked by means of the Shapiro-Wilk test and accordingly we transformed all variables by logarithmic function [*log*(x)], except LDMC for which we used the square root function [*sqrt*(x)]. To highlight the main adaptive trends within the trait space of our dataset and to seek relationships of sulfur traits (LSC, C/S) with these, we first performed a principal component analysis (PCA), centred by standard deviation and followed by varimax rotation, using the function 'principal' of the package 'psych' (Revelle 2018). The number of significant dimensions were identified by comparing the eigenvalues of each component with the value given by the broken stick distribution (MacArthur 1957), and statistically significant correlations between PCA axes and plant traits were identified using Pearson's correlation coefficient.

We applied one-way analysis of variance (ANOVA) with Tukey post-hoc comparisons to find differences of plant traits among the categories of life form, leaf life span and CSR plant strategies. In order to exclude influences of phylogenetic correlation we used linear mixed-effects models considering the family as a random effect. For each ANOVA, we tested the effect of family comparing the results of the linear mixed-effects model with those of a general linear model without any random effect (Dalle Fratte et al. 2019a). The addition of family on the models was always highly significant ($p < 0.01$). We also applied simple one-way ANOVA with Tukey post-hoc comparisons to test differences of plant traits among plant families, considering only those represented by at least 10 species.

We used simple linear regression models to evaluate the log-log relationship of LSC (dependent variable) with LNC (independent variables) within the whole dataset, as well as within each single category of life form, leaf life span, CSR plant strategies, and within each plant family (represented by at least 10 species). The log-log regression has been evaluated as a robust method for estimating nutrient ratios (Isles 2020), and has been showed to provide insight concerning nitrogen and sulfur acquisition in relation to plant strategies (Legay et al. 2014; Wu et al. 2017). We considered the R^2 to assess the amount of variation explained by each regression model, and to identify the relation of the ratio between LSC and LNC among plant families and CSR plant strategies, we correlated the slope of the linear regression (LSC vs. LNC) with the average values of family's CSR scores. We used the package ‘nlme’ (Pinheiro et al. 2018) for the linear mixed-effects model, the base R package ‘stats’ for general linear model and simple linear regression, and the package ‘ggtern’ (Hamilton and Ferry 2018) for the ternary visualization of CSR strategies.

3. Results

The main principal adaptive trends of species in the PCA were represented by a two-dimensional space (cumulative percentage of variance = 69 %, Fig. A2) defined by variation in the LES (PC1 = 45 %) and of the size and the dimensions of leaves and plant height (PC2 = 24 %; Fig. 1). The first axis (PC1) correlated positively with SLA and LNC, and negatively with LDMC and C/N (Table 1), indicating variation in trait values ranging from acquisitive strategies to values indicating conservative resource use, while PC2 correlated positively with CANH, LDW and LA (i.e. towards taller plants with larger and heavier leaves). Sulfur traits (LSC and C/S) correlated significantly with PC1 and grouped, respectively, with acquisitive and conservative traits.

The LES was thus well defined by six traits, including LSC and C/S, which also showed statistically significant differences among life form categories (Fig. 2). Trees and shrubs exhibited the lowest mean values of all the acquisitive traits (SLA, LNC and LSC), while short-lived herbs displayed the highest mean values of SLA and LSC. Only for LNC, short and long-lived herbs did not exhibit significant differences among each other. With regard to the set of conservative traits, trees and shrubs showed the highest mean values of C/N and C/S, while trees displayed significantly higher values of LDMC compared to shrubs. Short-lived herbs were the less conservative, showing the lowest LDMC and C/S, but similar values of C/N compared to long-lived herbs. With regard to leaf life span categories, evergreen species were more conservative compared to deciduous and wintergreen, which were indeed more acquisitive.

Only a few plant families exhibited significant differences of the mean values of traits correlated with the LES (Fig. A3). With regard to leaf traits, such as SLA and LDMC, the *Pinaceae* family was found to be the most conservative, while *Orchidaceae*, *Brassicaceae* and *Caryophyllaceae* were the most acquisitive, despite not showing significant differences compared to many families with intermediate characteristics. Nutrient traits confirmed *Pinaceae* and *Brassicaceae* to be families that were significantly different from others, respectively placed at the conservative and acquisitive extremes of the LES. Nitrogen and sulfur traits showed a similar pattern, even though LNC and C/N discriminated more the *Pinaceae*, while LSC and C/S the *Brassicaceae*.

LSC was significantly higher for all plant strategy categories with a high ruderal (R) or competitive (C) component: CSR, CR, C, R (Fig. 3b). Specifically, the R strategy showed on average the highest LSC values, in contrast to S-selected species, which showed the lowest values, only comparable to those of the SC category. Only a few species, among those with higher LSC, were placed toward the centre of triangle (*Erysimum rhaeticum* and *Mercurialis annua*) or even toward the S-selected corner (*Triglochin palustris*, *Equisetum fluviatile*, *E. variegatum*) (Fig. 3a).

LSC showed a significant positive linear relationship with LNC (Table 2) considering all species together (slope = 0.81, $R^2 = 0.30$, $p < 0.01$), which was even more robust within the R strategy category (slope = 0.95, $R^2 = 0.42$, $p < 0.01$). With regard to families, we found an increase of both slope and R^2 compared to the overall equation for *Orchidaceae*, *Poaceae*, *Juncaceae*, *Rosaceae*, and *Caryophyllaceae*. Finally, we observed a positive correlation between the mean R scores and the slope values of the linear regression equations LSC vs. LNC calculated for each plant family (Fig. 4). *Caryophyllaceae* and *Orchidaceae* were the two families with the highest mean R scores and consequently showed the highest slope values (LSC vs. LNC), while *Apiaceae* and *Salicaceae* were found at the opposite extreme of this gradient.

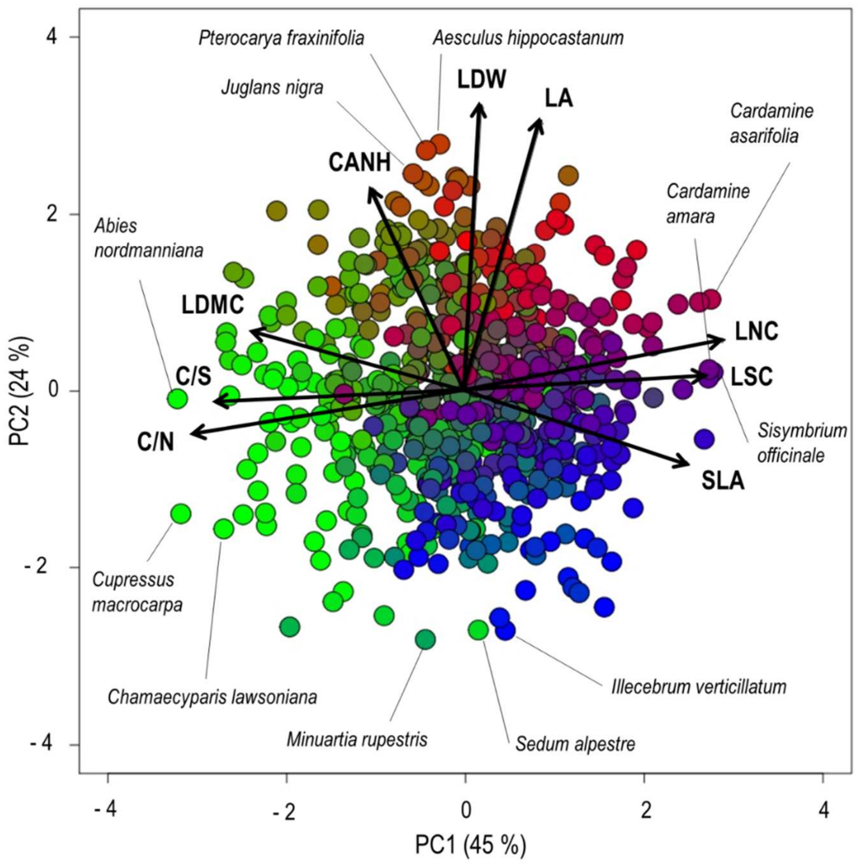

Figure 1: Principal Component Analysis on traits values of the 740 vascular plant species selected for the analysis. Species points are coloured by RGB classification according to their CSR scores calculated with *StrateFy* (Pierce et al., 2017). Labelled points are species with the three highest and lowest values on PC1 and PC2. Legend: CANH = canopy height, C/N = carbon to nitrogen ratio, C/S = carbon to sulfur ratio, LA = leaf area, LDMC = leaf dry matter content, LDW = leaf dry weight, LNC = leaf nitrogen content, LSC = leaf sulfur content, SLA = specific leaf area.

Table 1: Pearson's correlation coefficients (r) of the first two principal component analysis (PCA) axes with traits values for the 740 plant species selected for the analysis. Emboldened values are highly correlated ($r > \pm 0.5$) and statistically significant at $p \leq 0.01$ level (critical value of r = 0.09, df = 739). Legend: CANH = canopy height, C/N = carbon to nitrogen ratio, C/S = carbon to sulfur ratio, LA = leaf area, LDMC = leaf dry matter content, LDW = leaf dry weight, LNC = leaf nitrogen content, LSC = leaf sulfur content, SLA = specific leaf area.

	PC1	**PC2**
LA	0.25	**0.90**
LDW	0.05	**0.95**
CANH	-0.31	**0.67**
SLA	**0.73**	-0.24
LNC	**0.85**	0.17
LSC	**0.79**	0.05
LDMC	**-0.70**	0.20
CN	**-0.89**	-0.14
CS	**-0.81**	-0.04

Table 2: Coefficients of simple linear regressions between LSC (dependent variable) and LNC (independent variable) for all species and different categories of life form, leaf life span, Grime's CSR strategies, as well as for those family represented by at least 10 species in the dataset. Emboldened values are those with R^2 higher than the one of the overall equations. Legend: α = intercept, β = slope, p = p-value (ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

		α	β	R^2	p
ALL SPECIES		-2.37	0.81	**0.30**	***
LIFE FORM	**tree**	-2.36	0.56	0.21	***
	shrub	-2.35	0.71	0.25	***
	long-lived herb	-2.38	0.82	0.29	***
	short-lived herb	-2.12	0.73	0.22	***
LEAF LIFE SPAN	**deciduous**	-2.22	0.63	0.19	***
	wintergreen	-2.43	0.93	**0.32**	***
	evergreen	-2.46	0.59	0.15	**
CSR STRATEGIES	**C**	-2.29	0.84	0.20	**
	CR	-2.43	0.90	0.23	***
	CSR	-2.20	0.60	0.12	***
	R	-2.41	0.95	**0.42**	***
	SR	-2.36	0.84	0.21	***
	S	-2.36	0.68	0.22	***
	SC	-2.25	0.57	0.14	***
FAMILY	***Pinaceae***	-2.54	0.97	0.25	*
	Asparagaceae	-2.40	0.75	**0.55**	**
	Orchidaceae	-3.48	1.54	**0.52**	**
	Poaceae	-2.51	1.08	**0.54**	***
	Juncaceae	-2.29	1.01	**0.40**	**
	Cyperaceae	-2.21	0.59	0.17	***
	Salicaceae	-1.58	0.20	0.01	ns
	Ranunculaceae	-2.15	0.54	0.10	ns
	Leguminosae	-2.75	0.85	0.21	**
	Rosaceae	-2.78	0.90	**0.46**	***
	Brassicaceae	-1.74	0.86	0.20	*
	Caprifoliaceae	-2.28	0.69	**0.38**	*
	Caryophyllaceae	-2.81	1.18	**0.64**	***
	Lamiaceae	-2.05	0.49	0.18	ns
	Plantaginaceae	-1.94	0.54	0.17	ns
	Compositeae	-2.32	0.90	0.21	***
	Apiaceae	-1.63	0.06	0.00	ns

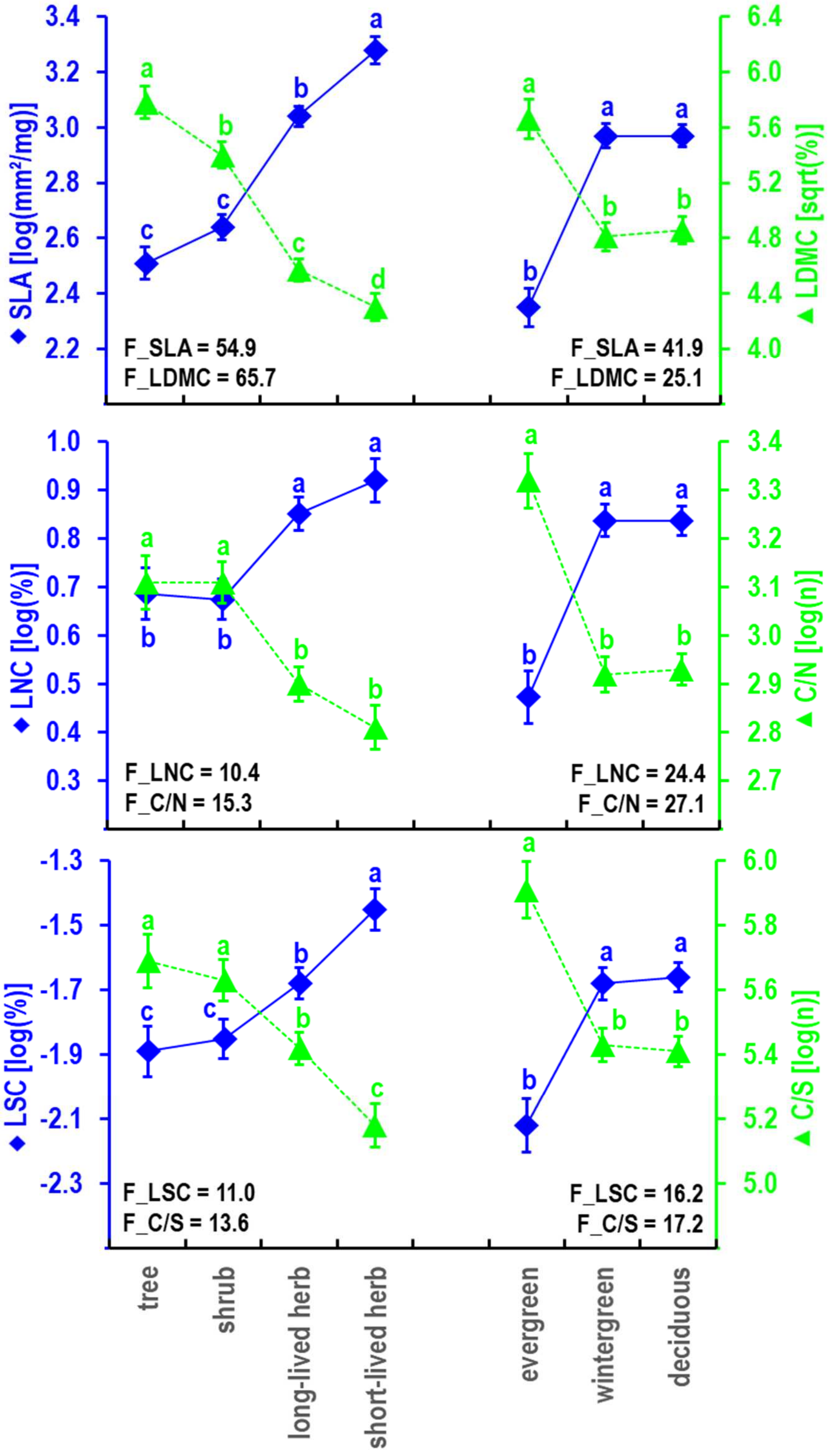

Figure 2: Mean values and standard error (vertical lines) of traits involved in the LES (Leaf Economics Spectrum) for each life form and leaf life span category. Small letters represent post-hoc comparison. All the ANOVAs were highly significant ($p < 0.001$, F-value reported in the figure). Legend: C/N = carbon to nitrogen ratio, C/S = carbon to sulfur ratio, LDMC = leaf dry matter content, LNC = leaf nitrogen content, LSC = leaf sulfur content, SLA = specific leaf area.

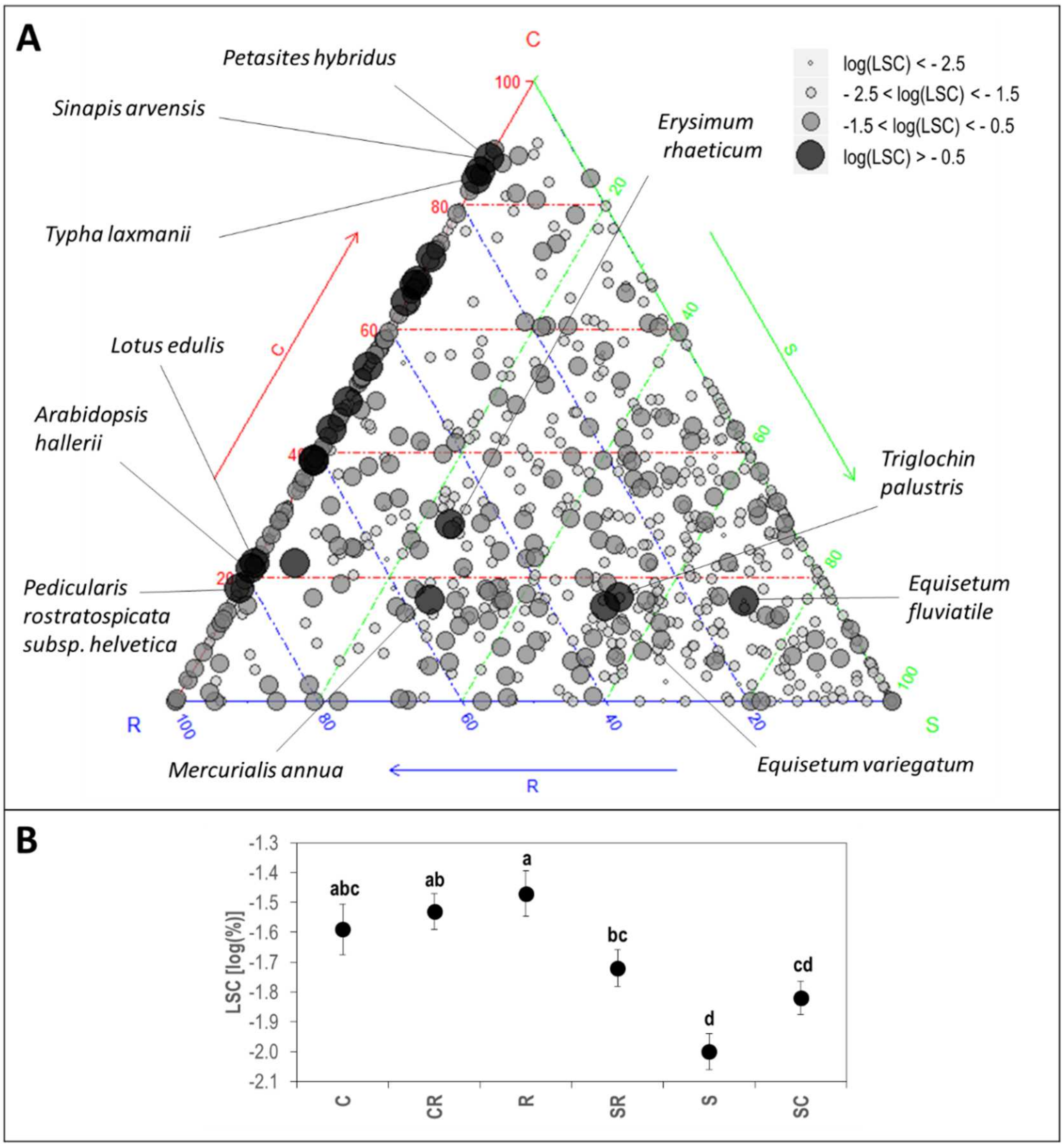

Figure 3: (A) Ternary visualization of CSR (Competitive, Stress-tolerant, Ruderal) strategies of the 740 species selected for the analysis; darker and wider points denote higher values of LSC (Leaf Sulfur Content). (B) Mean values and standard error (vertical lines) of LSC among plant strategies categories (F = 11.8, $p < 0.0001$). Small letters represent post-hoc comparison.

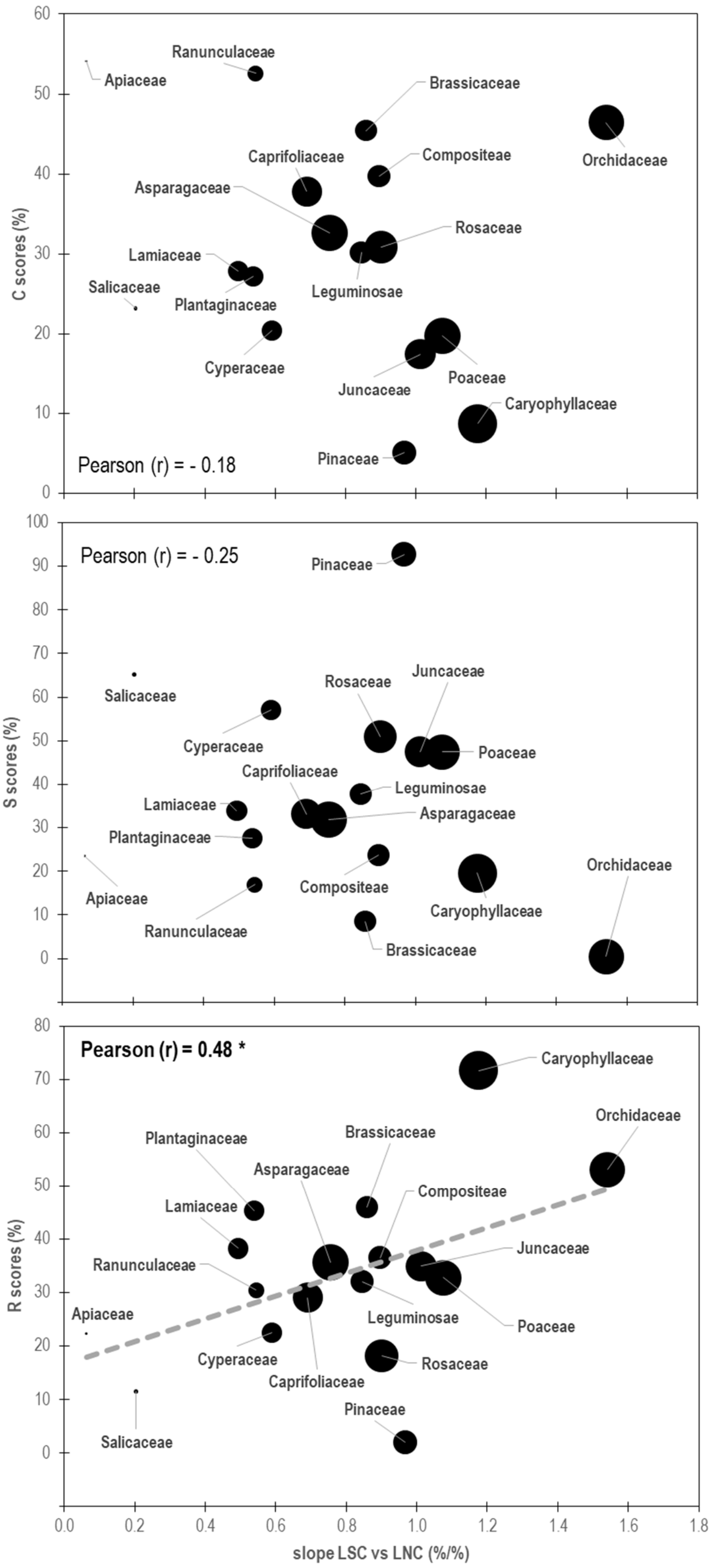

Figure 4: Relationship between slope values resulted from the linear regression LSC vs LNC within plant families represented by at least 10 species, and their mean values of C-, S- and R- strategies scores. Larger points denote higher R2 values of the linear regression LSC vs LNC (Table 2). Pearson's correlation coefficients (r) is reported in each figure; the emboldened one is significant at $p \leq 0.05$ (critical value = 0.48, df = 15).

4. Discussion

Firstly, our analysis at the regional scale confirmed the robustness of the two main axes of variation of plant functioning: representing the LES (i.e. fast-slow leaf economics; Reich 2014), and the size and dimensions of plant and leaves (both comprising the global spectrum of plant form and function; Díaz et al. 2016; Fig. 1 and Table 1). The pattern of SLA, LNC, together with LSC at one extreme and of LDMC, C/N, together with C/S at the other, reflected, respectively, the acquisitive-to-conservative trade-off determining the LES (Wright et al. 2004, Reich 2014), demonstrating that variation in LSC was associated with variation in the LES, supporting Hypothesis 1. Indeed, in our study the LES was the dimension accounting for the largest source of variance. The main reason for this apparent discrepancy with respect to the global spectrum may be related to the lower sample size but also to the different set of traits used in our analyses and, specifically, the inclusion of sulfur traits. This leads to the principal finding of our work, namely that LSC and C/S are in strong agreement with variation in the traits normally used for global spectrum studies, given their evident contribution to the LES axis. This also suggests that additional nutrients other than carbon, nitrogen and phosphorous, less frequently included in functional trait analyses, may also be embroiled in the LES (Sardans et al. 2008, Laliberté et al. 2012, De La Riva et al. 2018). Plant growth requires at least 17 mineral elements used in leaves to support fundamental physiological processes (Marschner 2012). Accordingly, SLA and leaf nutrient contents are supposedly closely related across species (Niinemets and Kull 2003, Wright and Cannon 2001, Wright et al. 2004).

We observed a significant linear relationship between LSC and LNC that confirmed previous similar results (Legay et al. 2014, Wu et al. 2017, Dijkshoorn and Van Wijk 1997; Table 2), highlighting that the assimilation processes of sulfur and nitrogen are associated (Hasanuzzaman et al. 2018), as most available reduced nitrogen and sulfur are incorporated into amino acids and subsequently into proteins (De Kok et al. 2017). Nitrogen together with phosphorous are integral to proteins of the photosynthetic apparatus, including Rubisco, and their contents are thus positively correlated with net CO_2 assimilation rate, dark respiration rate and relative growth rate (Wright et al. 2004, Reich et al. 2008). In addition to chloroplasts formation (Scherer 2008), sulfur has specific roles in fundamental processes, from photosynthesis to carbon and nitrogen metabolism (Droux 2004), including roles not shared by nitrogen and phosphorous but relevant to key biochemical pathways: for example, it contributes to ferredoxin oxidation (De Kok et al. 1993), and is part of Iron-sulfur clusters that aid the production of protein sufB, required for chlorophyll production (Hu et al. 2017). Sulfur thus represents an additional limitation to chlorophyll content and photosynthetic capacity with respect to nitrogen contents (Terry 1976, Resurreccion et al. 2001), and is vital to the achievement of the higher photosynthetic and respiration rates that characterize acquisitive species (Wright et al. 2004).

In contrast, conservative species exhibit greater mechanical support (e.g. high LDMC) and low photosynthetic and respiration rates, which involves greater leaf construction costs and times (Wright and Cannon 2001, Wright et al. 2004). C/N and C/S provide an indication of the relative investment in structure (carbon) and cell functioning (nitrogen and sulfur) and thus represent alternative measures of resource economics with respect to SLA and LDMC (Freschet et al. 2010), which use just mass measurements, also of clear physiological relevance. Indeed, physiological rates such as photosynthetic capacity and respiration rate are "carbon traits" (as opposed to "water or nutrient traits"; Reich 2014) that determine the amount of organic (carbon-based) matter a plant can produce. Species having leaves with a higher C/N are usually slow growing (Pierce et al. 2007, Freschet et al. 2010) with higher lignin content, and this optimization has also been found to relate to defence against herbivory (Hanley et al. 2007, Moreira et al. 2018). Thus, the addition of C/S to this framework could aid predictions concerning plant resistance to herbivory, as it expresses not only leaf digestibility (which corresponds with high leaf nutrient contents) but it also informs with regard to plant toxicity provided by sulfur-based plant defence metabolites (e.g. Badanes-Perez et al. 2014).

Leaf digestibility can subsequently alter litter decomposability (Cornelissen et al. 2004). Indeed, C/N has often been used as proxy of environmental variables such as soil organic matter quality and litter decomposition rates (Freschet et al. 2010, 2012). As a rule, carbon-to-nutrient ratios are important determinants of whether an element will be immobilized or released as litter decomposition proceeds (Blair 1988). Below a critical threshold, nutrients will be incorporated in soil microbial biomass and by-products as carbon are mineralized, thus lowering carbon-to-nutrient ratios. Plant community composition and soil fertility interact through complex feedbacks (Aerts and Chapin 1999, Wardle et al. 2004) that are detachable by traits related to the LES (Cornwell et al. 2008, Bakker et al. 2011, Freschet et al. 2012). Based on our findings, it is possible to extend the same interpretation of C/N, in terms of litter decomposability, also to C/S. Accordingly, LSC could influence litter decomposition rates by altering the structure of microbial communities (Legay et al. 2014, Pressler et al. 2020) as well as fungi development (Schowalter et al. 1998). LSC has therefore a significant connection to soil carbon dynamics (Miatto and Batalha 2016, Shi et al. 2016) and to nutrient economy at the community level (Laliberté et al. 2012), which was recently found to reflect the variation from communities with resource-acquisitive (high leaf nitrogen and phosphorous content) to those with resource-conservative characteristics (high LCC) (Bruelheide et al. 2018).

Leaf structure is considered one of the main drivers of the LES (Wright et al. 2004) and plant functional types have often been invoked to explain differences existing along the LES (Reich 2014, Díaz et al. 2016). In this context, we detected significant differences classifying species into broad functional types, that is, within both life form and leaf life span categories. Trees and shrubs, as well as evergreen species, showed higher affinities for conservative strategies, in contrast to short and long-lived herbs, as well as wintergreen and deciduous species, which overall demonstrated relatively acquisitive strategies. This interpretation was supported by differences in mean values of all analysed leaf traits, including sulfur traits (Fig. 2). Evergreen species, compared to wintergreen and deciduous, require greater mechanical support, so that the increase of LDMC and the corresponding decrease of SLA are related to a greater portion of carbon in structural tissue (Villar and Merino 2001), which makes them less susceptible to environmental hazards and stress (Bussotti et al. 2005, Sardans et al. 2008, Poorter et al. 2009). They also displayed lower LNC and LSC, associated with higher C/N and C/S, indicating low nutrient requirements due to a more efficient use, typical of slow growing species (De La Riva et al. 2018), despite the greater energetic costs of tissue construction (Villar and Merino 2001).

We observed similar large differences in traits related to the LES among woody and non-woody life forms, confirming that herbs, particularly short-lived herbs, are more acquisitive than trees and shrubs. Our results show once more that the use of life forms can help to discriminate variations along the gradients underlying the LES (Wright et al. 2005, Thomas et al. 2019) and nutrient use efficiency, as herbaceous species, particularly short-lived ones, are those with higher leaf nutrient contents (Han et al. 2011), sulfur included. Nevertheless, at the global scale woody and herbaceous species have shown extensive overlap along the LES (Díaz et al. 2016), partially due to the larger number of species and range of climates that were considered in the global study. However, we found only herbaceous species at the most acquisitive extreme of the LES, confirming their adaptation to include relatively acquisitive traits (Pierce et al. 2013), as opposed to trees, which instead have a more conservative set of traits.

We found evidence that high levels of LSC strongly relate to the ruderal strategy (the extent of R-selection) and, to a lesser extent, to the competitive strategy (C-selection), while the stress tolerant strategy (S-selection) was characterized by the lowest values, confirming the predictive strength of Grime’s CSR adaptive strategies also regarding nutrient economics (Pierce et al. 2007) (Fig. 3). Only a few species with a high LSC were located towards the stress-tolerant (S-selection) corner; specifically, two species of the genus *Equisetum* (*E. fluviatile* and *E. variegatum*). This is not surprising if we consider that *Equisetaceae* are known to have high nutrient contents (Marsh et al. 2000) due to an efficient nutrient uptake that allows these species to thrive under a wide range of conditions (Husby 2013). Moreover, they exhibit an abnormally high accumulation of silica, up to 25 % of dry weight (Gierlinger et al. 2008) that, combined with the development of photosynthetic stems, may provide heavier structures and hence apparent affinities to stress-tolerant strategies. Among all the CSR strategies categories, ruderal species showed the closest linear relationship between LSC and LNC ($R^2 = 0.42$) and the steepest slope ($\beta = 0.95$), i.e. the greatest portion of sulfur compared to nitrogen (Table 2). This led us to hypothesize that the ratio of the two nutrients may be more stable within this subset of species, which must invest in a rapid growth to complete faster the lifecycle, in order to maintain the population in the face of disturbances events (Grime 2006, Grime and Pierce 2012, Pierce et al. 2017).

Leaf nutrient contents are also linked to taxonomy (Zhang et al. 2012, Miatto and Batalha 2016), as we clearly observed for the *Pinaceae* (and other conifers) and for the *Brassicaceae*, respectively placed at the conservative and at the acquisitive extremes of the LES (Fig. 1 and Fig. A3). *Brassicaceae* displayed the highest values of LSC, probably because of the production of sulfur-containing glucosinolates (Dijkshoorn and Van Wijk 1997, Badanes-Perez et al. 2014). They also showed high values of LNC, which was very generally but significantly correlated with LSC ($R^2 = 0.20$, $p < 0.05$; Table 2), probably because both nutrients are employed to various extents throughout primary and secondary metabolism. A tighter linear relationship between LSC and LNC (i.e. higher values of R^2; Table 2) was instead shown by other families with a higher degree of ruderality than *Brassicaceae*, like *Orchidaceae* and *Caryophyllaceae*, which also showed a higher LSC to LNC ratio (i.e. higher values of the slope β; Table 2). This evidence strengthens the overall linear relationship found for ruderal strategies, but also suggests that, considering families, the greater it is the extent of R-selection, the more the portion of sulfur compared to nitrogen in leaves is high and stable (Fig. 4). Explaining high values of LSC, and their constancy in relation to LNC, is beyond the scope of the present study, and likely involve many other products of the secondary metabolism involving sulfur compounds (Hawkesford and De Kok 2007), for example sulfur-based floral volatiles and blends associated with orchid pollination (Wong et al. 2017).

The biological stoichiometry of plants can play a key role in exploring evolutionary processes and adaptive variation in the biota (Kay et al. 2005), thus is crucial in evaluating ecological responses to global change (Elser et al. 2010). We observed high relevance of sulfur for plant functioning that can be integrated with other plant traits (Bernhardt-Römermann et al. 2008) to learn more about the processes and patterns of ecosystem development in response to environmental changes. Future changes of nutrient deposition loads, together with climate change, will determine new environmental scenarios that may substantially alter the chemical composition of terrestrial ecosystems (e.g. Zhan et al. 2017), with profound consequences for competition among species (Tallec et al. 2008), plant community composition (Boutin et al., 2017) and biogeochemical cycles (Shi et al. 2016). Specifically, the biogeochemical cycle of sulfur is (and has been) largely impacted by anthropogenic activities so that it is necessary to understand its contribution within plant functioning and how it could affect the kind of species (i.e. with fast or slow economics) that can grow in a given environment. However, few LSC data appear to be available today in international trait databases (e.g. in TRY, Kattge et al. 2020), compared to other leaf nutrient contents, particularly LNC. In light of this, here we presented a large dataset of leaf traits, including LNC and LSC as relevant, covering a wide range of plant forms and functions, useful to integrate, at least at the regional scale, the understanding of plant functional responses to global change (see Chelli et al. 2019).

5. Conclusions

The link identified here between sulfur traits, LSC and C/S, and other leaf traits underlying the LES provides insights concerning the role of sulfur for plant functioning. The interaction between sulfur and other nutrients, specifically LNC and C/N, suggests that sulfur traits also scale up to ecosystem properties, such as biomass production or litter decomposability, which are strictly related with LES traits. We also observed a positive correlation between LSC and LNC, which showed different patterns throughout broad functional type categories. Among these, Grime's CSR adaptive strategies were found to be the most reliable, demonstrating their wide applicability also in connecting knowledge of sulfur physiology to plant ecology. Specifically, we found that species with high LSC were associated with the acquisitive extreme of the LES detected at the global scale, which was specifically represented by highly R-selected (ruderal) strategies.

6. References

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7. Supplementary material

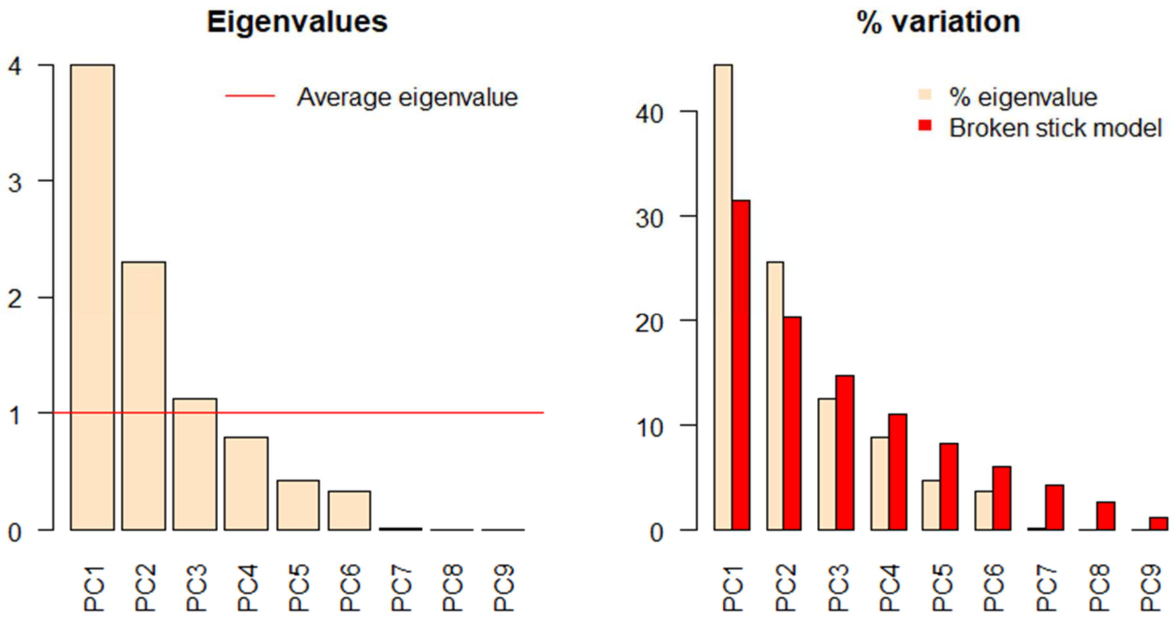
Appendix A (Table A1): List of all plant families considered in the analysis (number of records = 740). Emboldened families are those represented by at least 10 species, selected for the regression analysis between LSC (Leaf Sulfur Content) and LNC (Leaf Nitrogen Content).

Family	n°
Poaceae	**75**
Cyperaceae	**70**
Compositae	**66**
Rosaceae	**40**
Leguminosae	**39**
Apiaceae	**23**
Caryophyllaceae	**21**
Brassicaceae	**19**
Pinaceae	**17**
Juncaceae	**16**
Plantaginaceae	**16**
Caprifoliaceae	**13**
Lamiaceae	**13**
Orchidaceae	**13**
Ranunculaceae	**13**
Salicaceae	**13**
Asparagaceae	**12**
Cupressaceae	9
Fagaceae	9
Rubiaceae	9
Saxifragaceae	9
Betulaceae	8
Crassulaceae	8
Oleaceae	8
Amaryllidaceae	7
Campanulaceae	7
Gentianaceae	7
Onagraceae	7
Orobanchaceae	7
Ericaceae	6
Malvaceae	6
Polygonaceae	6
Adoxaceae	5

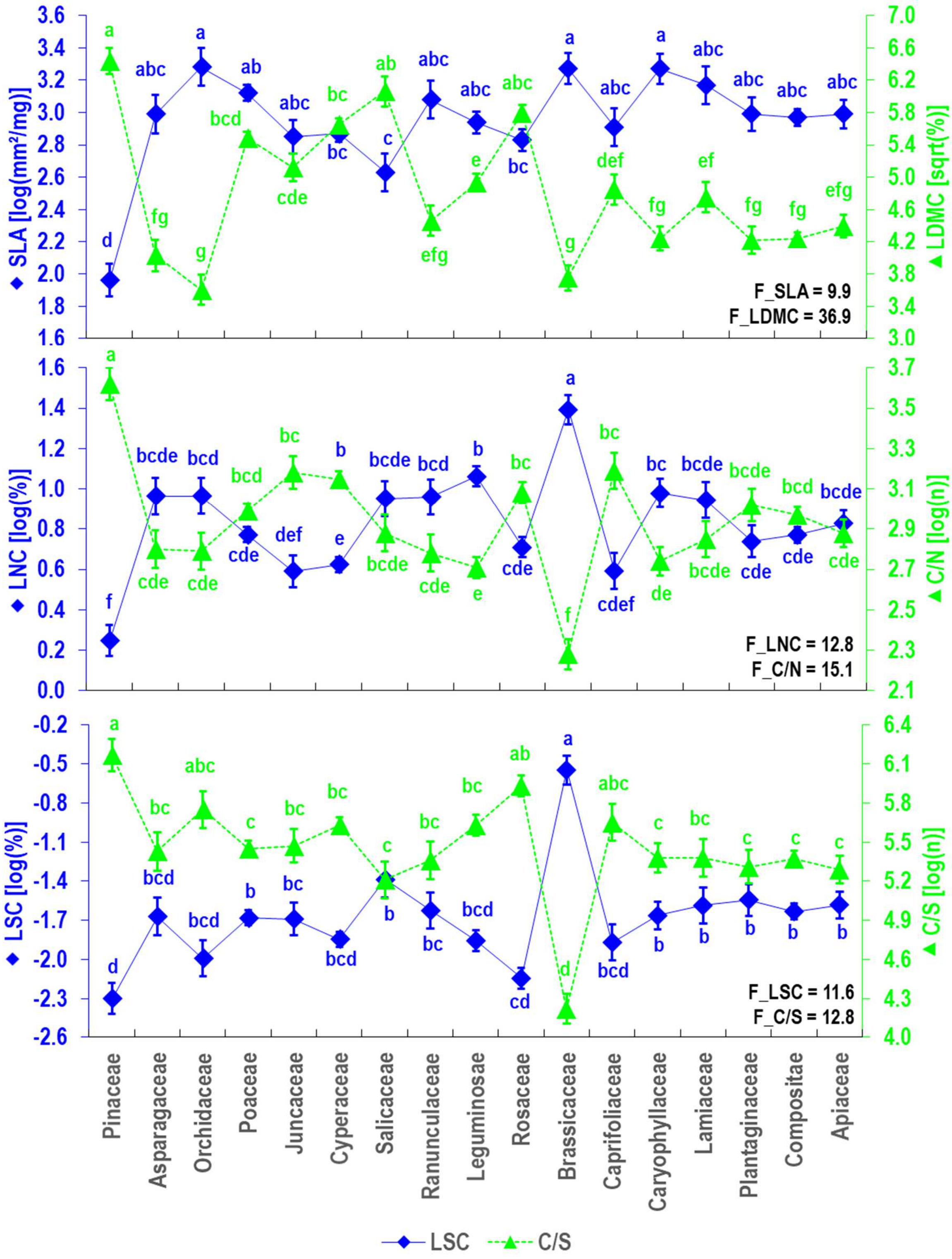
Family	n°
Aspleniaceae	5
Boraginaceae	5
Ulmaceae	5
Violaceae	5
Equisetaceae	4
Liliaceae	4
Magnoliaceae	4
Papaveraceae	4
Primulaceae	4
Scrophulariaceae	4
Apocynaceae	3
Convolvulaceae	3
Cystopteridaceae	3
Droseraceae	3
Euphorbiaceae	3
Geraniaceae	3
Lentibulariaceae	3
Linaceae	3
Lythraceae	3
Sapindaceae	3
Solanaceae	3
Typhaceae	3
Xanthorrhoeaceae	3
Alismataceae	2
Anacardiaceae	2
Araceae	2
Balsaminaceae	2
Cannabaceae	2
Cistaceae	2
Ebenaceae	2
Hydrangeaceae	2
Hypericaceae	2
Juglandaceae	2

Family	n°
Lycopodiaceae	2
Moraceae	2
Oxalidaceae	2
Pteridaceae	2
Rhamnaceae	2
Santalaceae	2
Taxaceae	2
Actinidiaceae	1
Altingiaceae	1
Amaranthaceae	1
Aristolochiaceae	1
Berberidaceae	1
Bignoniaceae	1
Butomaceae	1
Cornaceae	1
Cucurbitaceae	1
Elaeagnaceae	1
Ginkgoaceae	1
Iridaceae	1
Juncaginaceae	1
Lauraceae	1
Menyanthaceae	1
Myrtaceae	1
Nyctaginaceae	1
Paulowniaceae	1
Pittosporaceae	1
Platanaceae	1
Plumbaginaceae	1
Polygalaceae	1
Rutaceae	1
Scheuchzeriaceae	1
Smilacaceae	1
Verbenaceae	1

Appendix B (Figure A2): Eigenvalues of each component of the PCA (left), and their comparison with expected eigenvalues, computed following the broken stick model (MacArthur, 1957). Accordingly, a component is retained if its associated eigenvalue is larger than the value given by the broken stick distribution.



Appendix C (Figure A3): Mean values and standard error (vertical lines) of traits involved in the LES (Leaf Economics Spectrum) among plant families represented by at least 10 species. Small letters represent post-hoc comparison. All the ANOVAs were highly statistically significant ($p < 0.001$, F-value reported in the figure). Legend: C/N = carbon to nitrogen ratio, C/S = carbon to sulfur ratio, LDMC = leaf dry matter content, LNC = leaf nitrogen content, LSC = leaf sulfur content, SLA = specific leaf area.



Plant trait variation along environmental indicators to infer global change impacts

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Flora 254: 113–121
https://doi.org/10.1016/j.flora.2018.12.004

Abstract

One of the key principles for modeling future impacts of anthropogenic and climate changes on vegetation is to identify clear patterns and trends between plant functional traits and ecological drivers. The global spectrum of plant form and function outlines the major axes of variation and coordination among plant traits. We hypothesized that inter-specific plant trait variation at the regional scale should match axes of plant adaptation highlighted by the global spectrum, and that plant trait–environmental associations should be evident over ranges of environmental indicators corresponding to key drivers of future climate and land use changes in Southern Europe. To test our hypotheses, we analyzed a sample of 1095 plant species from Northern Italy, also characteristic of Southern European vegetation. We analyzed trends for four plant traits (canopy height, leaf area, specific leaf area, leaf nitrogen content) along meso- (temperature, continentality) and micro climatic (light) ranges, as well as along soil ranges (moisture, reaction and nutrient content), by means of Landolt's environmental indicators. At the regional scale, the inter-specific variation of mean plant traits over ranges of environmental indicators confirmed the main axes of plant adaptation emphasized by the plant global spectrum. Leaf area and specific leaf area showed the largest sensitivity over ranges of all environmental indicators, while canopy heights were the most responsive to temperature values. Temperature, light conditions and nutrients were associated with clear effects on plant traits, underlining that responses to changes in land use and increased soil nutrient loading (sudden and abrupt changes) could trigger and strengthen responses to climate alteration (gradual changes). Scenarios for the next few decades in Southern Europe indicate increased temperature, nutrient availability and forest coverage that, according to our findings, will favor more 'acquisitive and fast growing' plants, also represented by subtropical invasive species.

Keywords: Plant global spectrum, Ecological gradients, Landolt, Climate change, Land use change

1. Introduction

In the Anthropocene epoch, human activities have changed the global climate, land cover and biodiversity at unprecedented rates, exacerbating negative effects on plant diversity (Parmesan 2006; Walther et al. 2002). Worldwide impacts of global change are a cause of concern for future scenarios in plant diversity alteration (Parmesan and Hanley 2015), and its cascade effects on ecosystem functioning and the provision of services (Chapin et al. 2000; Naeem et al. 2009). In Southern Europe, in future decades a rise in temperatures and eutrophication are expected due to an increase in nitrogen deposition, while the concomitant increase in forested areas will lead to an overall decline in light availability (EEA 2017a, 2017b; Kovats et al. 2014; Leip et al. 2015; Rounsevell et al. 2006; Tilman et al. 2001).

Responses of plant communities to climate change depend strongly on the functional ecology of species (Suding et al. 2008), an aspect of their biology that has been increasingly investigated in recent decades, thanks to an increasing number of studies on Plant Traits (PTs) (Garnier et al. 2017). PTs are among the best proxies used to observe plant responses to changes in environmental conditions (Lamarque et al. 2014; Vandewalle et al. 2010). They integrate the ecological and evolutionary history of a species, thereby reflecting trade-offs among different functions within a single plant and determining species ecological roles in the environment (Violle et al. 2007). PTs influence plant fitness via their effects on resource acquisition, growth, reproduction and survival (Dıaz and Cabido 2001). Indeed, they quantify the functional dimensions of plants and are fundamental to detect their ecological strategies (Grime and Pierce 2012; Pierce et al. 2017).

Extensive studies focused on the plant economics spectrum found evidence that support the ‘traits manifesto’ (Reich, 2014). Globally the two main axes of functional variation in vascular plant traits (Diaz et al., 2016) are those related to the size of organs and whole plant, and those associated with leaves, the leaf economics spectrum (Wright et al. 2004). The first dimension denotes the ability of plants to make use of light resources, disperse diaspores, and their colonization ability in space and time. It runs from short and small-leaved species tending to have small diaspores to tall and broad-leaved species tending to have large diaspores, broadly reflecting the r (colonization) versus K (exploitation) continuum. The second dimension indicates how plants acquire and use resources. It represents the variation from large leaves with high specific leaf area and leaf nitrogen content, to small leaves with low specific leaf area and leaf nitrogen content, which is a tradeoff among ‘fast and acquisitive’ to ‘slow and conservative’ growth.

PTs have been shown to vary along ecological gradients for at least some sites and combinations of species (e.g., Ackerly et al. 2002; Herben et al. 2018; Shipley et al. 2017). Nevertheless, there is still no clear consensus about which PTs best predict climate change responses (Parmesan and Hanley

2015). The links with a single ecological driver have only rarely been systematically evaluated, since it requires a large sampling effort (Rosbakh et al. 2015), although it is a necessary step toward an accurate mapping of current or future spatial distribution of PTs (Butler et al. 2017; van Bodegom et al. 2014; Wright et al. 2017). Selection pressures of defined ecological drivers should generate predictable variation of PTs following the main axes of adaptation within the plant economics spectrum, at least at the inter-specific level (Vellend et al. 2014), providing useful opportunities to explore the ability of plants to respond to global changes.

In this context, Environmental Indicators (EIs) (Ellenberg et al. 1992; Landolt et al. 2010) could be suitable, since they are scores for each single species representing their average position within the range of key ecological drivers. They have been criticized since they are derived from the personal observations of authors and do not rely on objective measurements (Shipley et al., 2017). However, they have a long history of validation (e.g., Klaus et al. 2012; Scherrer and Korner 2011; Thompson et al. 1993; Wamelink et al. 1998), so they may provide an early warning signal of changes in the environment. For this reason, they have been widely applied in studies of vegetation ecology as well as studies concerning global changes (Diekmann 2003; Scherrer and Korner 2011). In the context of the plant's competitive situation, PTs should vary accordingly to EIs, depending on the environmental filters, since EIs represent coordinates of plants' niche in natural communities as defined by their physiological growth limits (Landolt et al. 2010). Hence, insights from the analysis of mean values of PTs along EIs ranges could reconcile two main fields of ecology. This could provide a deeper understanding of plant functional responses to future climate and land use changes, especially considering trends in PTs and their responses at the extremes of EI ranges.

In this study, we evaluated the extent of inter-specific variation of key PTs of the plant global spectrum (Diaz et al. 2016) along a range of ecological drivers. To this end, we studied a large sample of vascular plants form Southern Europe, and estimated ecological drivers using the EI scores of the study species. Firstly, we hypothesized that inter-specific variation of PTs at the regional scale should match the major axes of plant adaptation highlighted by the plant global spectrum (Diaz et al. 2016). Secondly, variation over ranges of EIs should correspond to PT variation along gradients of key ecological drivers of global changes (temperature, rainfall, light and N availability, soil reaction and moisture).

2. Methods

2.1 Plant traits (PTs)

In accordance with the plant global spectrum (Diaz et al. 2016), we selected two "size" traits (Canopy Height and Leaf Area, respectively CANH and LA), and two traits linked to the "leaf economics spectrum" (Specific Leaf Area and Leaf Nitrogen Content per leaf dry mass, respectively SLA and LNC). PT data were selected from Authors datasets available in TRY (Kattge et al. 2011; https://www.try-db.org/ see datasets n. 227, 228, 229, 371, 372 and related references), augmented with some previously unpublished data from our own database.

For each species, within the same population we sampled from 5 to 15 fully expanded leaves selected randomly from the outer canopy of different individual adult plants growing under optimal conditions, following a standardized methodological protocol (Perez-Harguindeguy et al. 2016). We measured CANH as the maximum vegetative height: distance between the ground and the highest vegetative leaves of the plant (i.e. excluding inflorescences; Perez-Harguindeguy et al. 2016). For this measure, we selected the tallest individual of each species among those identified for leaf sampling. Plants were measured in their normal state, i.e. without stretching them. CANH was quantified in situ, while all the other PTs were determined from analysis of material collected in the field and analysed in the laboratory. For LNC we processed three replicates (each from a single individual shoot) from dry leaf material with a CHNS-analyzer (FlashEA 1112 series Thermo-Scientific). More detailed methodological protocol concerning collection of leaf material and sample processing procedures are reported in Cerabolini et al. (2010).

Sampling sites (n=154) are widespread over an area of approximately 89,000 km2, covering 22 provinces of Northern Italy. Almost all records of our dataset (96%) are representative of vegetation types of Alpine (Southern Alps) and Continental (Po Plain) biogeographic zones (ETC/BD 2006) (Fig. 1). Except for some samples from the Mediterranean biogeographic region, the climate ranges from continental to oceanic regimes (Pesaresi et al. 2014), although the complex morphology of the study area results in considerable variability of mesoclimates, from warm climates close to the main lakes, to middle-European of lowland areas and to cold Alpine regimes of high elevation. Substrates vary from silicatic or carbonatic rocks to sedimentary deposits of various sources, with additional extensive variability at the local scale.

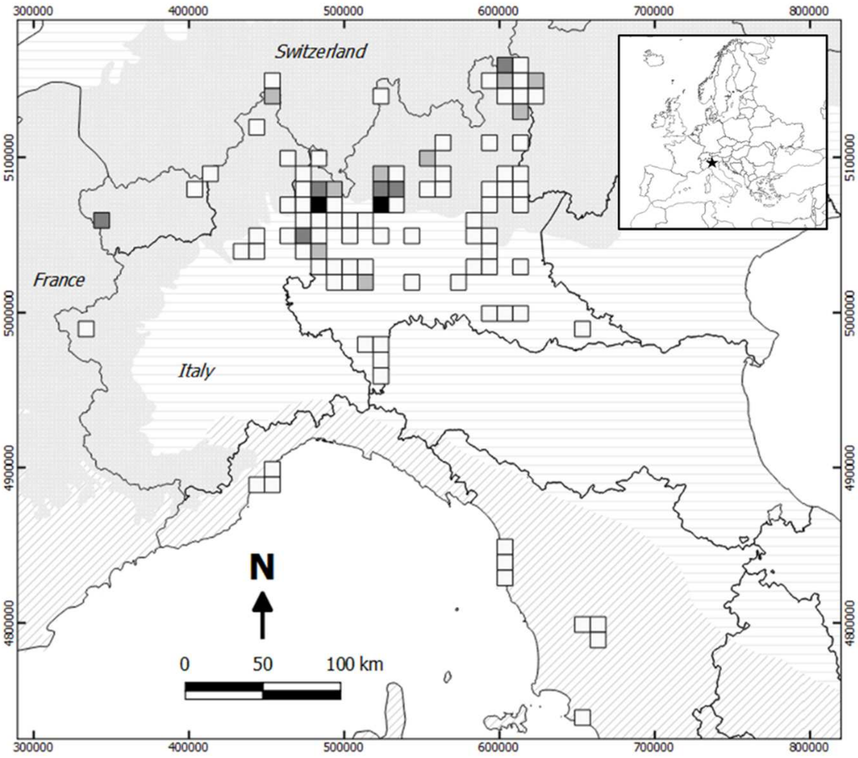

Figure 1: Map of the spatial distribution of the analysed dataset (reference system WGS84/UTM 32 N). The 10 km×10 km grid has been categorized using the sum of all records contained in each pixel: 1–15 (white), 16–30 (light grey), 31–50 (dark grey), and 51–150 (black). The black line indicates the administrative borders, i.e. borders of France and Switzerland, and the administrative regions of Italy. Biogeographic regions (ETC/BD, 2006) are indicated: Alpine=dotted grey; Continental= strikethrough horizontal grey, Mediterranean=strikethrough diagonal grey. The black star in the inset map indicates the location of the study area.

2.2 Environmental indicators (EIs)

EIs for the vascular plants of Central Europe (see Ellenberg et al. 1992) have also been determined for many floras of adjacent regions. Those published for the Swiss flora have a long tradition culminating with the 2010 edition of the Flora Indicativa (Landolt et al. 2010), that we considered the most suitable to describe species distribution along ranges of ecological drivers in our study area, since it neighbours Switzerland (Fig. 1).

For each species, we considered two meso-climatic (temperature, T; continentality, K), one micro-climatic (light, L) and three soil indicators (moisture, F; reaction, R; nutrients N), allocating them to indicators' classes as reported in Flora Indicativa (Landolt et al., 2010). T characterises the average air temperature during the growth period of a plant, which largely corresponds with the average elevational distribution of species. It ranges from alpine and nival (1) to very warm colline (5). K indicates whether the air is humid and the daily and annual variation of temperature occur within small ranges (small values), or the air is dry and there is a large variation in temperature (high values). It ranges from oceanic (1) to continental (5). L specifies the average light quantity received in the respective habitats. It ranges from deep shade (1) to full light (5). Concerning soil indicators: F is the average soil moisture during the growth period; it ranges from very dry (1) to flooded (5); R is the content of free H-ions in the soils, it ranges from extremely acid (1) to alkaline (5); N is the nutrient content in the soil, referring mostly to nitrogen but also to phosphorus, and spreads from very infertile (1) to very fertile (5).

2.3 Data analysis

We selected 1095 species from 50 families (Peruzzi 2010), excluding species of underrepresented families in the overall dataset (i.e. with less than five species); thus the dataset submitted to analysis included 108 trees, 76 shrubs, 209 graminoids and 702 forbs (Appendix A). LNC analysis were performed on a smaller dataset, due to the lack of available data (838 taxa analyzed), while hydrophytes were excluded from CANH analysis (1057 taxa analyzed). We also excluded species indifferent to a single EI, marked as “x” in Landolt et al. (2010), from analysis concerning that EI (Appendix B). However, the proportion of “x” species was always small, ranging from 0% (L and N) to 1.2% (LNC vs T). According to Landolt et al. (2010), each EI has its own scale of indicator values, mainly a unit increment ranging from 1 to 5. In T and F the 1 to 5 range is split into 0.5 unit increments, resulting in 9 classes. To be able to compare the results among EIs, we assigned species with “intermediate” values to the next unit class toward the extremes of the range (i.e. 1.5 to 1, 2.5 to 2, 3.5 to 4, and 4.5 to 5). Data were first normalized by logarithmic (LA, CANH and SLA) or square root (LNC) transformation, and normality checked according to the Shapiro Wilkman test. We compared differences in PTs among the five classes of each EI by means of analysis of variance (ANOVA). We used a linear mixed-effects model, considering the family as a random effect to exclude influences of phylogenetic correlation. For each combination of PTs vs EIs, we tested the effect of family via ANOVA, comparing the results of the linear mixed-effects model with those of a general linear model, considering only the classes of EIs as factors. The addition of family on the models was always highly significant ($p < 0.01$). Post-hoc comparisons on the results of linear mixed-effects models were then calculated by Tukey's test. For PTs showing statistically significant differences following post-hoc comparison, we estimated the regression trend for the means of PTs along the classes of EIs using linear or non-linear regression (quadratic) models. We selected the most significant regression trend comparing the two models through ANOVA. We then compared the effect sizes of EIs and their interactions on each PT through a multifactorial ANOVA with interactions, using eta squared (η2) to quantify the effect sizes (Levine and Hullett, 2002). All the statistical analyses were computed with R software (R Core Team, 2017)**3. Results**

Canopy height (CANH)

With regard to meso- and micro-climatic EIs (Fig. 2), CANH showed highly significant differences in terms of T (F4,995=111.7, $p < 0.001$) and L (F4,1006=65.7, $p < 0.001$) ranges, while differences were less distinct for K (F4,1003=4.8, $p < 0.001$). Mean canopy heights were significantly shorter in species of the lowest temperature regimes (T=1) and increased progressively reaching the maximum in species of higher temperature regimes (T=4 and T=5). Mean canopies showed an overall significant linear decrease along the L range: they were higher in moderate shade classes (L=2 and L=3) and lower in well-lit (L=4) and mostly in full light (L=5) classes. On the other hand, deep shade species displayed a slightly opposing trend, but represented a small proportion of the dataset (about 0.2%) (Appendix B), and this may have affected the outcome.

Among soil EIs (Fig. 3), CANH exhibited significant differences only over the R (F4,995=24.2, $p < 0.001$) and the N (F4,1006=46.3, $p < 0.001$) ranges, since we found weak significant differences among F classes (F4,1001=2.4, p=0.05) with no significant post-hoc comparisons ($p > 0.05$). However, while the CANH differences over R classes did not show a clear trend, over the N range they showed a statistically significant non-linear increase, consisting of a saturation in the two higher N classes of fertile and over-rich soils (N=4 and N=5). The lowest mean canopy heights were those of very infertile soils (N=1).

Leaf area (LA)

Mean LA showed significant differences over all the climatic EI (Fig. 2) ranges: T (F4,1030=36.8, $p < 0.001$), K (F4,1038=2.9, p=0.02) and L (F4,1041=54.0, $p < 0.001$). Leaves were on average significantly smaller in species of the lowest temperature regimes (T=1) and progressively larger in warmer classes, showing a slight decrease in the warmest one (T=5). Mean LA was also significantly higher in species of intermediate moisture climates (K=3) but a clear pattern of leaves size was not detectable towards the extremes of the K range. Concerning light availability, along L range mean LA progressively decreased moving from shade to full light conditions (i.e. from L=2 to L=5), although the trend was not significant. Outcomes may be affected by the shortage of deep shade species in the dataset, as mentioned above.

LA displayed highly significant differences over all the soil EI ranges (Fig. 3): F (F4,1036=16.2, $p < 0.001$), R (F4,1030=12.5, $p < 0.001$) and N (F4,1041=67.0, $p < 0.001$), although the evident non-linear layouts highlighted along F and R ranges by post-hoc comparisons were not supported by significant regressions. Mean LA was smaller in species growing in dry and very dry soils (F=1) and in wet and flooded soils (F=5), if compared to moist soils species (F=4). At the same time, mean LA was significantly smaller in species growing in acid (R=2) and alkaline soils (R=5) but larger for species from soils at intermediate reaction (R=3 and R=4). A clear progressive increase of mean LA was evident throughout the range from very infertile (N=1) to very fertile and over-rich soils (N=5).

Specific leaf area (SLA)

Mean SLA showed highly significant differences along all the meso and micro-climatic EI ranges (Fig. 2): T (F4,1030=13.2, p < 0.001), K (F4,1038=14.8, p < 0.001) and L (F4,1041=37.2, p < 0.001). Leaves were on average tougher (low values of SLA) in species from low temperature regimes of high elevation (T=1 and T=2) compared to those from warmer climates of the montane and colline belts (T=3 and T=4). Species proper of warmest climates (T=5) showed a slight decrease of mean SLA, and they were significantly different only from those of the colline belt (T=4). Nevertheless, the trend of mean SLA over the T range was not significant.

Mean SLA values were significantly higher in species from climates under oceanic influence (from K=1 to K=3) and lower in those linked to continental regimes (K=5), as underlined by the highly significant non-linear trend. Mean SLA values decreased linearly as light availability increased, being higher in species growing in shade (L=2) and lower in those linked to full light sites (L=5). The slight decrease of mean SLA in deep shade species (L=1) can likely be due to the shortage of deep shade species in the dataset, as mentioned above. Mean SLA also displayed significant differences throughout all the soil EI ranges (Fig. 3): F (F4,1036=26.0, p < 0.001), R (F4,1030=12.3, p < 0.001) and N (F4,1041)=27.4, p < 0.001). Species linked to very dry soils (F=1) showed the lowest value of mean SLA, which increased linearly in species growing in moderate dry soils (F=2) and again in those from moderately moist to flooded soils classes (F=3, F=4, F=5), but with no further differences. Along the R range, the highest value of mean SLA occurred in species of the intermediate class representing almost neutral soils (R=3), while comparable low mean SLA values belonged to species of extremely acid (R=1) or alkaline soil (R=5) classes. Mean SLA values clearly increased alongside nutrient availability, until an asymptotic saturation appeared from medium fertile to over-rich soils (N=3, N=4, N=5).

Leaf nitrogen content (LNC)

Among the climatic EI ranges (Fig. 2), mean LNC showed significant differences over T (F4,774=2.9, p=0.02) or L (F4,784=7.9, p < 0.001) classes, while no significant differences were found over K classes (F4,782=1.9, p=0.11). However, mean LNC values did not display a significant trend along T range and the post-hoc comparisons showed significant differences only between species of two classes (T=3 vs T=5). On average, mean LNC values were significantly higher in species linked to moderate shade (L=2, L=3) compared to those assigned to well-lit and full light classes (L=4, L=5), resulting in a linear decreasing trend of mean LNC as light availability rose. Species of deep shade did not show any differences.

Conversely, mean LNC displayed significant differences along all the soil EI ranges (Fig. 3): F ($F_{4,781}=14.8$, $p < 0.001$), R ($F_{4,777}=4.1$, $p < 0.003$) and N ($F_{4,784}=14.1$, $p < 0.001$). It was significantly lower in species linked to dry soils (F=1), then increased until saturation in species linked to classes of F indicating moderate moist to flooded soils (from F=3 to F=5). Along the R range, a non-linear trend was readily detectable, although in the post-hoc comparisons significant differences emerged only between species from neutral soils (R=3) and those linked to alkaline soils (R=5), the first ones showing a higher mean LNC value. Ultimately, mean LNC values revealed a significant marked linear increase along N range, with lower values belonging to species of very infertile and infertile soil classes (N=1, N=2) and higher values in species linked to very fertile and over-rich soils (N=5).

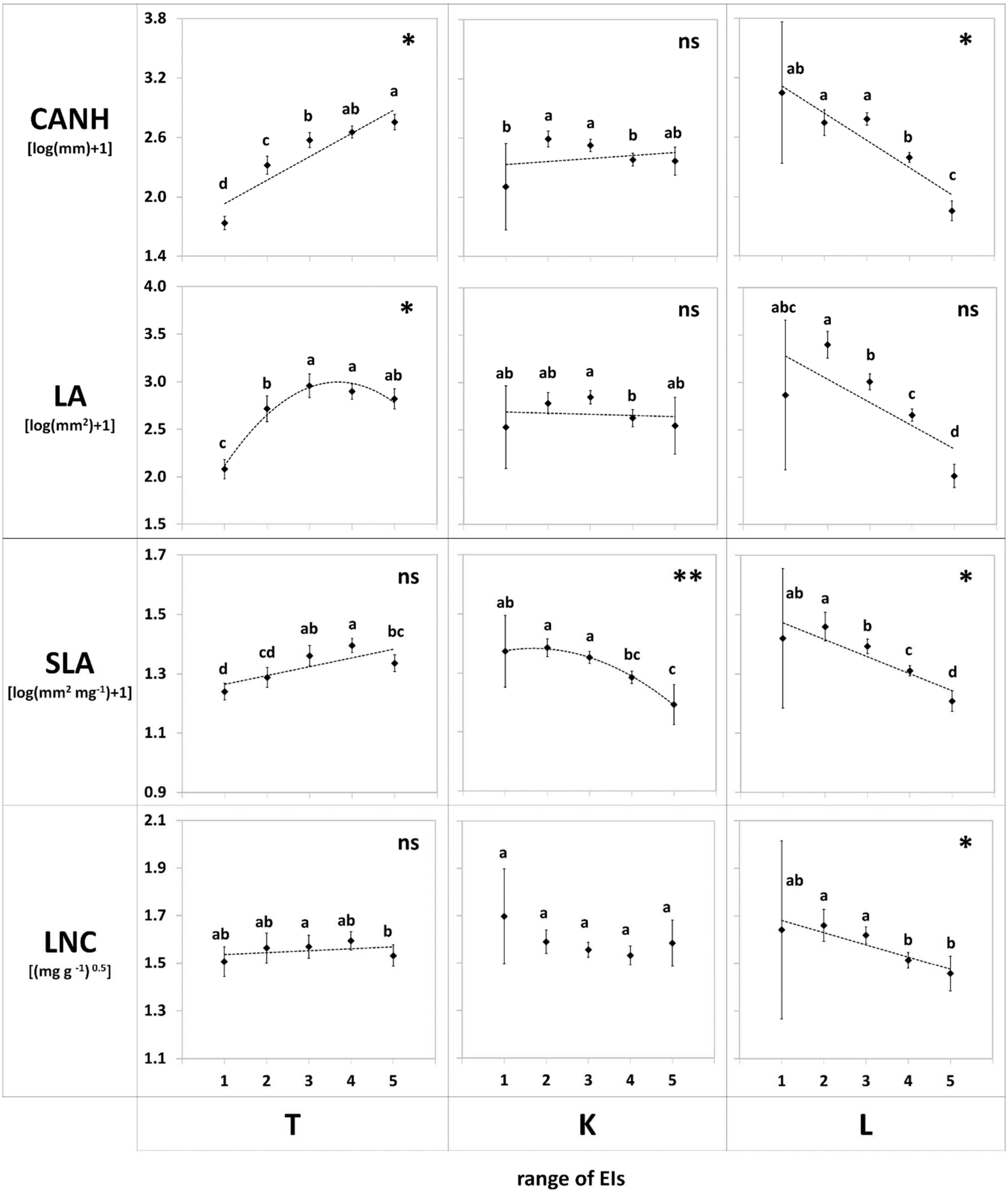

Figure 2: Mean (± 1.96*SE) of Plant Traits–PTs (CANH=Canopy Height, LA=Leaf Area, SLA=Specific Leaf Area, LNC=Leaf Nitrogen Content) vs climatic Environmental Indicator classes–EIs (T=Temperature, K=Continentality, L=Light) according to Landolt et al. (2010). Lower-case letters indicate the results from Tukey post-hoc comparison following ANOVA. Regression trends between PTs and EIs are plotted only when ANOVA with Tukey post-hoc comparison was statistically significant: (ns) p-value>0.05; (*) p-value ≤ 0.05; (**); p-value ≤ 0.01.

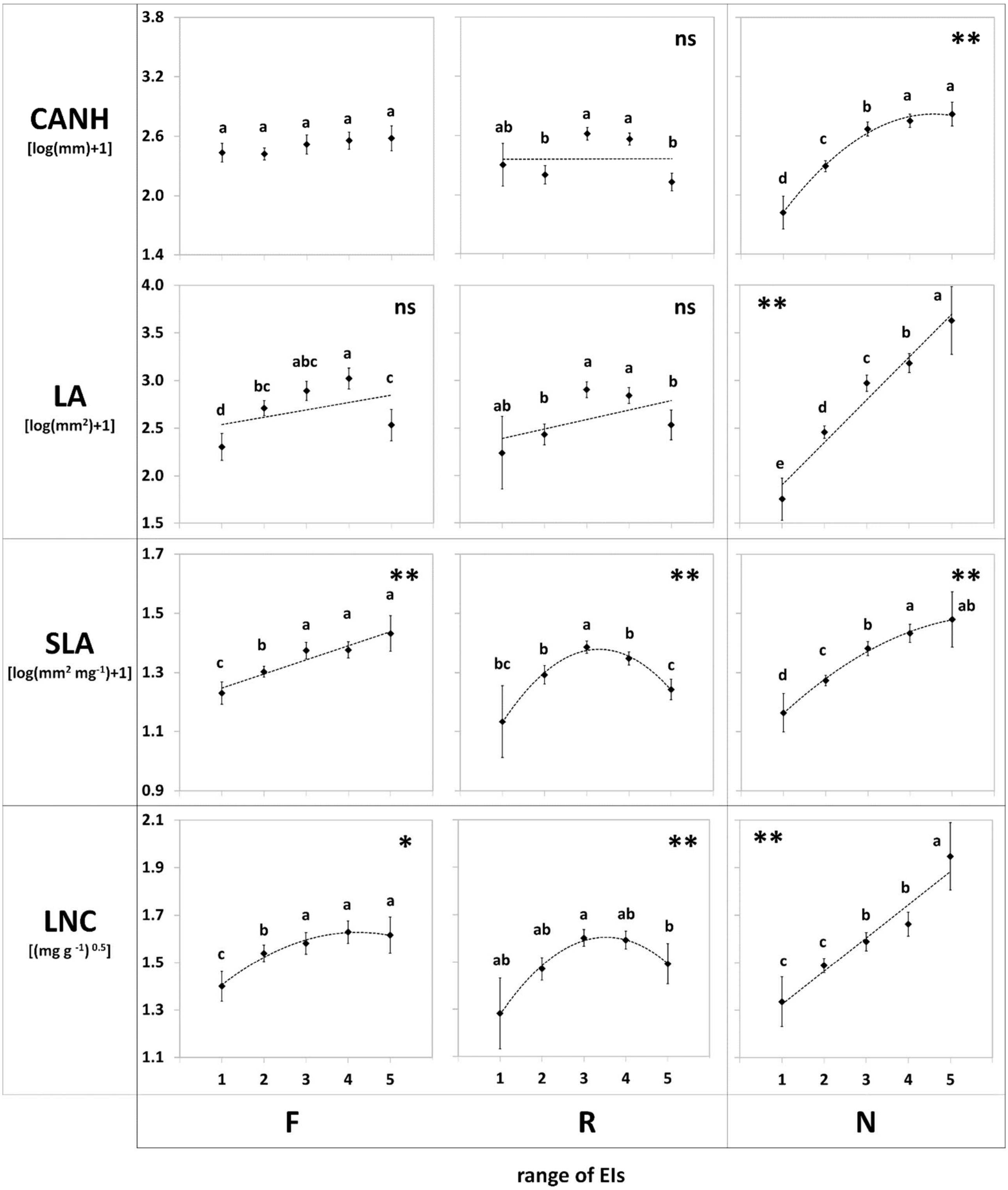

Figure 3: Mean (± 1.96*SE) of Plant Traits–PTs (CANH=Canopy Height, LA=Leaf Area, SLA=Specific Leaf Area, LNC=Leaf Nitrogen Content) vs soil conditions Environmental Indicator classes–EIs (F=Moisture, R=Reaction, N=Nutrients) according to Landolt et al. (2010). Lower-case letters indicate the results from Tukey post-hoc comparison following ANOVA. Regression trends between PTs and EIs are plotted only when ANOVA with Tukey post-hoc comparison was statistically significant: (ns) p-value>0.05; (*) p-value ≤ 0.05; (**); p-value ≤ 0.01.

4. Discussion

Our analyses, based on a large dataset, broadly confirmed at the regional scale of Southern Europe the main axes of adaptation of vascular plants determined by the global spectrum of plant form and function (Diaz et al. 2016). Whole plant size, represented by CANH, and organ size, represented by LA, showed similar responses and consistent trends throughout all the EI ranges, although with slightly different levels of significance. Similarly, but more evidently, SLA and LNC varied over EI ranges in agreement with the leaf economics spectrum (Wright et al. 2004). At a broad scale, we observed a general response of PTs to environmental associations depicted by Landolt's EIs. Among these, we found subtle patterns of PT variation associated with continentality (K) while the most remarkable patterns concerned soil nutrient content (N). In a very schematic way we can state that, 'size' PTs (CANH and LA) were influenced more by meso- and micro-climatic EIs (T and L), while PTs representing the leaf economics spectrum (SLA and LNC) exhibited evident responses to soil EIs (F, R and N). However, the occurrence of synergic effects due to shifts in environmental drivers should always be taken into account, although our analysis concerning the interactions between couples of EIs mainly exhibited no significance or small effect sizes (Table 1). On the other hand we must consider that environmental drivers will likely act in an asynchronous way, since climate warming may occur more gradually compared to more abrupt changes in land use, especially with regard to local soil nutrients loadings (eutrophication), so that the former will act on vegetation assets modified by the latter. We are well aware of the critical points of our broad approach that need to be followed up by more detailed and deeper analyses to validate and to fine-tune our outcomes. For example, differential responses over EI ranges may be expected by dividing the traits dataset used here among growth forms (Shipley et al. 2017) or plant strategies (Pierce et al. 2017), or further considering intra-specific traits variation along better defined gradients (Vellend et al. 2014) to gain insights with regard to species' chances of acclimatization when faced with changing ecological conditions. *4.1. Climate indicators* Our data showed the largest effect size scores in relation to the T range for CANH and LA (Table 1), evidence that agrees with the fact that temperature rise will undoubtedly be the major environmental driver of climate change determining future vegetation distributions. From low to high temperature regimes we found, in accordance with previous studies, groups of species with higher mean canopy height (Sandel et al. 2016; Tardella et al. 2016) and bigger mean size of leaves (Hodgson et al. 2011; Scoffoni et al. 2011; Wright et al. 2017). Temperature regimes had much less effect on traits linked to the leaf economics spectrum, even though we recorded a mean SLA increase from low to high T classes: i.e. passing from 'slow and conservative' to 'fast and acquisitive' leaves (Borgy et al. 2017; Fontana et al. 2017; Rosbakh et al. 2015; Sandel et al. 2016; Wright et al. 2004). We can therefore assume that warmer climate expected in Southern Europe in future decades (EEA 2017a; Kovats et al. 2014) will facilitate large-leaved species with taller canopies, and that high elevation vegetation of the Southern Alps will also be affected, as recent findings across the tundra biome have highlighted (Bjorkman et al. 2018). Despite this, at the warmest extreme of the T range, LA, SLA and LNC showed a moderate decrease that fits well with the "laurophyllisation" phenomenon of lower vegetation belts in Southern Switzerland (Walther et al. 2002), although leaf life span was not included in our dataset. Over the continentality range mean values of traits were the least responsive, probably because of the small amplitude of this range in Switzerland, obscured by the attribution of Mediterranean species to the medium class instead of to the driest one (Landolt et al. 2010). Although an increase of plant height together with precipitation was found for grasslands (Sandel et al. 2016), species in our dataset showed higher mean values in intermediate classes, as observed by Fontana et al. (2017). Our outcomes also cannot corroborate the evidence that broad-leaved species are associated with wetter climates (e.g. Hodgson et al. 2011; Scoffoni et al. 2011; Wright et al. 2017). Continentality range was associated with a significant decrease of mean SLA values (Table 1) in agreement with previous observations (Hodgson et al. 2011; Rosbakh et al. 2015; Sandel et al. 2016; Shipley et al. 2017; Wright et al. 2004). Accordingly, we can assume that species with more conservative leaves (low SLA) will be favored by the average precipitation decrease predicted in future scenarios (EEA 2017a; Kovats et al. 2014). The light availability indicator exhibited significant effects on all PTs (Table 1), which showed a general progressive decrease from shade to full light conditions. Our outcomes are in agreement with competition for light, as shaded habitats are normally associated with 'acquisitive and fast growing' species (Hodgson et al. 2011; Rosbakh et al. 2015; Wright et al. 2004) with taller canopies (Fontana et al. 2017; Tardella et al. 2016). The functional variability of species adapted to deep shade is probably due to the wide array of responses displayed by understory plants (e.g., Valladares et al. 2002). We must also consider that local floras typically include fewer species adapted to deep shade with respect to those living in higher light conditions. For example, considering the whole flora of Switzerland (n=6472; Landolt et al. 2010), only 0.5% of species are included in the deep shade class. However, distribution of mean LA values along the light availability range did not reveal a significant trend at the inter-specific level considered by our analysis. Leaf area shifts with light availability are probably related to both single species and growth forms (Ackerly et al. 2002). An almost stable pattern of LA along irradiance gradient was observed for herbs and shrubs, while graminoids and trees showed a remarkable decline as irradiance increased (Shipley et al. 2017). According to the scenario modeled for Southern Europe (Rounsevell et al. 2006), future decades should be characterized by an increase of forested areas at the expense of grasslands, so species that are large, 'acquisitive and fast growing' and/or shade tolerant, will be favored by future land use circumstances.

4.2. Soil indicators From very dry to moist soils, as expected (Reich 2014), we observed a gradual shift from 'slow and conservative' to 'fast and acquisitive' species, denoted by the trends of leaf economics PTs (i.e. from low to high SLA and LNC). Similar trends have already been reported in the literature (Fraser et al. 2016; Garnier and Navas 2012; Herben et al. 2018). However, the increases of mean values of SLA and LNC were evident only in the first part of the range until the mesic condition was reached, and beyond this point the increase of soil moisture did not provide any significant modification of leaf economics PTs. A comparable pattern was also shown by leaf area (Table 1) although this decreased on wet soils, probably due to the contribution of leafless species (see *Cyperaceae* and *Juncaceae*). Small leaves were thus associated with both extremes of soil moisture, according to Shipley et al. (2017). Regardless of local presence of water bodies (rivers, lakes, ponds, mires etc.) soil moisture availability is strongly linked to temperature and precipitation regimes, so that models performed for Southern Europe predict a decrease of soil moisture in future decades (EEA, 2017a; Kovats et al. 2014), which will presumably favour species with relatively conservative strategies. Over the soil reaction range (R) all the mean values of PTs generally exhibited a convex unimodal relationship, reaching maximum values in the group of species growing in neutral soils. This humped pattern highlights the harsh soil conditions experienced by plants living at both extremes of the range, which are associated with conservative and slow growing species (Garnier et al. 2016), smaller in height and in leaf size (trends of these two latter PTs were not significant). Although responses of plants to soil reaction range were clear, insights concerning future vegetation scenarios driven by this factor are difficult to assess since it presumably will not change rapidly in the near future, being strictly associated with the geological substrate. Larger plants are expected to be strongly linked to very fertile soils, considering that nitrogen and phosphorus availabilities (to which the N indicator mainly refers; Landolt et al. 2010) are the main limiting factors of plant growth (Gusewell 2004; Stevens et al. 2018). Hence an increase in soil nutrient availabilities can lead to a parallel increase of values in all the PTs studied here (Ackerly et al. 2002; and references therein). Specifically, from poor to very rich soils plants exhibited consistently taller canopies (Tardella et al. 2016; Herben et al. 2018) and larger leaves (McDonald et al. 2003; Shipley et al. 2017). The two 'size' PTs differed each other only in the highest N class, in which mean values of canopy heights reached saturation. Conservative strategies (Stress tolerant, *sensu* Grime; see Grime and Pierce 2012) are linked to nutrient poor soils, while relatively acquisitive strategies (Competitive and Ruderal, *sensu* Grime; see Grime and Pierce 2012) to nutrient rich soils (Fraser et al. 2016; Garnier and Navas 2012; Reich 2014; Wright et al. 2004). This was also clearly shown in our results concerning mean 'leaf economics' plant traits (SLA and LNC), along the entire N range. Since soil nitrogen and phosphorus loadings in Southern Europe will continue to increase in the future decades (EEA 2017b;

Leip et al. 2015; Tilman et al. 2001), we can expect a rise of larger 'acquisitive and fast growing' species, with an overall enhancement of Competitive strategies and competition rates in plant communities. This could put a strain on biodiversity at the local scale (Bobbink et al.,2010) according to the rates of eutrophication or nitrogen deposition.

Table 1: Effect sizes (eta squared, η2) and significance (p-value) of each environmental indicator, and their interactions, on plant traits (CANH=Canopy Height, LA=Leaf Area, SLA=Specific Leaf Area, LNC=Leaf Nitrogen Content) as inferred by multifactorial ANOVA with interactions.

	CANH		**LA**		**SLA**		**LNC**	
	η^2	**p-value**	**η^2**	**p-value**	**η^2**	**p-value**	**η^2**	**p-value**
T	**0.269**	***	**0.124**	***	**0.052**	***	0.009	ns
K	**0.016**	***	**0.016**	***	**0.028**	***	0.003	ns
L	**0.065**	***	**0.090**	***	**0.043**	***	**0.037**	***
F	**0.012**	***	**0.052**	***	**0.027**	***	**0.033**	***
R	0.003	ns	**0.017**	***	**0.021**	***	**0.029**	***
N	**0.015**	***	**0.064**	***	**0.016**	***	**0.041**	***
T*K	**0.019**	**	0.009	ns	0.012	ns	**0.026**	*
T*L	**0.012**	*	0.008	ns	**0.025**	**	**0.026**	*
T*F	0.012	ns	0.007	ns	0.013	ns	0.02	ns
T*R	0.011	ns	0.015	ns	0.013	ns	0.014	ns
T*N	0.008	ns	0.012	ns	0.012	ns	0.014	ns
K*L	**0.014**	*	0.011	ns	0.016	ns	0.016	ns
K*F	0.007	ns	**0.021**	**	0.017	ns	0.014	ns
K*R	0.006	ns	0.010	ns	0.006	ns	0.014	ns
K*N	0.004	ns	**0.013**	*	0.009	ns	0.008	ns
L*F	0.011	ns	0.009	ns	**0.018**	*	**0.028**	*
L*R	0.012	ns	0.002	ns	0.015	ns	0.017	ns
L*N	0.005	ns	0.005	ns	0.004	ns	0.006	ns
F*R	**0.015**	*	0.015	ns	0.011	ns	0.013	ns
F*N	0.014	ns	0.009	ns	0.014	ns	0.011	ns
R*N	0.005	ns	0.006	ns	0.005	ns	0.006	ns

5. Conclusions

Consistent with trait-based niche theory (Reich 2014), the tendency of species to share PTs is connected to their broad habitat affinities as well as to their ecological niche, which can be described by EIs, at least at a general level. This evidence provides an elementary tool to gain insight into plant functional responses to changes in ecological drivers (Garnier et al. 2016). Among the PTs considered in our work, SLA and LA were responsive to all the ecological ranges identified by Landolt's EIs; hence we suggest that they are very suitable for monitoring of global change impacts. CANH showed the highest sensitivity over the T range, suggesting that species with taller canopies will very likely be favored in warmer scenarios throughout all vegetation types of Southern Europe, until the upper vegetation belts of the Alps (e.g. Bjorkman et al. 2018). Alternatively, land use changes which lead to nutrient increases in soils showed clear-cut effects along both major axes of the global spectrum, so that we can expect larger 'acquisitive and fast growing' species to be favoured, driving a potential increase of total biomass (Gornish and Prather 2014; Timmermann et al. 2015), consequently changing the competition rates within plant communities. Our results also suggest that future global changes could promote the establishment of further growth forms and functional types, currently only scarcely represented or lacking in Southern European local floras (Walther et al. 2002). Moreover, the globalization of plant species is favouring the introduction of invasive alien species (Ciappetta et al. 2016; Najberek et al. 2017), particularly those native to areas under warmer climates which possess combinations of traits associated with rapid resource acquisition and growth (Gioria and Osborne 2014; Guo et al. 2018; van Kleunen et al. 2010). This is currently evident in the large number of subtropical invasive species that are already spreading in Southern Europe, including Italy, and threatening local plant diversity (Assini et al. 2010): e.g. *Humulus japonicus* Siebold & Zucc., *Reynoutria japonica* Houtt., *Berberis bealei* Fortune, *Pueraria lobata* (Willd.) Ohwi, *Catalpa ovata* G.Don, *Trachycarpus fortunei* (Hook.) H.Wendl.

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7. Supplementary material

Appendix A: List of all plant families considered in the analysis (number of records = 1095).

Family	N°	Family	N°
Asteraceae	136	*Euphorbiaceae*	11
Poaceae	108	*Violaceae*	11
Cyperaceae	74	*Saxifragaceae*	11
Rosaceae	68	*Boraginaceae*	10
Fabaceae	65	*Pinaceae*	10
Caryophyllaceae	45	*Betulaceae*	9
Ranunculaceae	44	*Geraniaceae*	9
Lamiaceae	40	*Cistaceae*	9
Brassicaceae	32	*Fagaceae*	9
Apiaceae	31	*Dryopteridaceae*	8
Plantaginaceae	29	*Hydrocharitaceae*	8
Caprifoliaceae	22	*Oleaceae*	8
Juncaceae	22	*Amaranthaceae*	7
Campanulaceae	20	*Onagraceae*	7
Salicaceae	20	*Amaryllidaceae*	7
Polygonaceae	19	*Aspleniaceae*	7
Primulaceae	17	*Sapindaceae*	6
Orobanchaceae	16	*Araceae*	6
Ericaceae	16	*Equisetaceae*	6
Rubiaceae	16	*Malvaceae*	6
Crassulaceae	14	*Adoxaceae*	6
Orchidaceae	14	*Typhaceae*	5
Asparagaceae	13	*Scrophulariaceae*	5
Gentianaceae	12	*Urticaceae*	5
Potamogetonaceae	11	*Papaveraceae*	5

Appendix B: Synthesis of the 1095 records included in the dataset (EI value from 1 to 5) or excluded (EI value = x) in the Plant Traits analysis – PTs (LA = Leaf Area, SLA = Specific Leaf Area, CANH = CANopy Height, LNC = Leaf Nitrogen Content) along the climatic (T = Temperature, K = Continentality, L = Light) and environmental (F = Moisture, R = Reaction, N = Nutrients) gradients determined by Environmental Indicators – EIs. Records are expressed as total number (n) or percentage of the total (%).

		T		***K***		***L***		***F***		***R***		***N***	
PTs	***EI value***	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
CANH	***1***	147	13.9	17	1.6	8	0.8	116	11.0	21	2.0	41	3.9
	2	155	14.7	247	23.4	81	7.7	406	38.4	190	18.0	470	44.5
	3	168	15.9	448	42.4	339	32.1	207	19.6	388	36.7	322	30.5
	4	329	31.1	322	30.5	503	47.6	250	23.7	366	34.6	208	19.7
	5	247	23.4	20	1.9	126	11.9	73	6.9	81	7.7	16	1.5
	x	11	1.0	3	0.3	0	0.0	5	0.5	11	1.0	0	0.0
	Total (1-5)	1046	99.0	1054	99.7	1057	100.0	1052	99.5	1046	99.0	1057	100.0
LA	***1***	148	13.5	21	1.9	8	0.7	116	10.6	21	1.9	41	3.7
	2	155	14.2	264	24.1	81	7.4	406	37.1	193	17.6	476	43.5
	3	173	15.8	460	42.0	361	33.0	207	18.9	402	36.7	342	31.2
	4	348	31.8	327	29.9	519	47.4	250	22.8	387	35.3	220	20.1
	5	260	23.7	20	1.8	126	11.5	111	10.1	81	7.4	16	1.5
	x	11	1.0	3	0.3	0	0.0	5	0.5	11	1.0	0	0.0
	Total (1-5)	1084	99.0	1092	99.7	1095	100.0	1090	99.5	1084	99.0	1095	100.0
SLA	***1***	148	13.5	21	1.9	8	0.7	116	10.6	21	1.9	41	3.7
	2	155	14.2	264	24.1	81	7.4	406	37.1	193	17.6	476	43.5
	3	173	15.8	460	42.0	361	33.0	207	18.9	402	36.7	342	31.2
	4	348	31.8	327	29.9	519	47.4	250	22.8	387	35.3	220	20.1
	5	260	23.7	20	1.8	126	11.5	111	10.1	81	7.4	16	1.5
	x	11	1.0	3	0.3	0	0.0	5	0.5	11	1.0	0	0.0
	Total (1-5)	1084	99.0	1092	99.7	1095	100.0	1090	99.5	1084	99.0	1095	100.0
LNC	***1***	85	10.1	15	1.8	7	0.8	91	10.9	16	1.9	29	3.5
	2	99	11.8	203	24.2	73	8.7	300	35.8	150	17.9	342	40.8
	3	150	17.9	350	41.8	309	36.9	163	19.5	308	36.8	272	32.5
	4	288	34.4	251	30.0	376	44.9	190	22.7	309	36.9	180	21.5
	5	206	24.6	17	2.0	73	8.7	91	10.9	48	5.7	15	1.8
	x	10	1.2	2	0.2	0	0.0	3	0.4	7	0.8	0	0.0
	Total (1-5)	828	98.8	836	99.8	838	100.0	835	99.6	831	99.2	2324	100.0

Alien plant species invade by occupying similar functional spaces to native species

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Flora 257: 151419
https://doi.org/10.1016/j.flora.2019.151419

Abstract:

Invasive alien species (IAS) are the subset of naturalized species that cause greater impacts on biodiversity, ecosystem functions and services. However, despite management actions and eradication plans, their expansion worldwide is seemingly unstoppable.

In this paper, based on a large dataset of 1039 records of IAS and native plant species representative of the extensive biogeographic diversity in Southern Europe, we tested the consistency of Grime' concept of CSR universal adaptive strategies (competitors, stress-tolerators and ruderals) to explain plant invasion across a broad elevation gradient. Accounting for phylogenetic relatedness, we tested two hypotheses. First, whether IAS occupy a different CSR space compared to native species. Second, whether the success of IAS at the regional scale is linked to higher degree of C- and/or R-selection, according to recent worldwide observations on naturalization success of alien species. We tested such hypotheses on different growth forms (trees, shrubs, long-lived herbs, short-lived herbs, hydrophytes) that are related to plant functional traits.

Except for trees, IAS and native plant species essentially occupied the same CSR space, indicating that IAS can occupy the same niches of native species. However, IAS exhibited mainly higher C-scores compared to natives, suggesting that IAS are more competitive and are associated with relatively productive habitats, involving that these sites are highly prone to invasion (and that native species in resource-poor habitats are less likely to experience pressure from IAS). Our findings confirm the utility of CSR classification to broadly represent, and explain, the invasion success of alien plant species at the regional scale. This approach emerges as a viable tool in assessment of IAS characteristics and for development of strategies and plans for their containment.

Keywords: Competition, Exotic Invasion, Plant functional types, Plant strategies, Universal adaptive strategy theory

1. Introduction

Species introduction outside their natural boundaries is a rapidly increasing phenomenon fostered by globalization processes (Meyerson and Mooney 2007; Guo et al. 2019). Despite the lack of problems caused by many of these species on introduction to new locations, some become invasive (invasive alien species, IAS) and represent one of the major anthropogenic threats to ecosystem integrity worldwide, with impacts on biodiversity, ecosystem functioning and services (Pejchar and Mooney 2009; Vila et al. 2011). In Europe, which has a long history of species introduction (Lambdon et al. 2008b), risk assessments, early detection and eradication plans, and management actions have been widely implemented (e.g. Regulation No 1143/2014 of the European Parliament). Nevertheless, the expansion rate of IAS continues to increase (Pyšek et al. 2017), damaging economic and social aspects of human well-being. Thus, understanding the ecological characteristics of IAS could provide supporting information to develop more effective measures for their containment.

Among plant species, IAS are recognised as those that rapidly broaden their spatial distribution by expanding into existing native plant communities (Richardson et al. 2000). Although invasions start from natural or human induced dispersal processes, a range of biological and physical factors can provide the opportunity for IAS to rapidly outcompete native species (Rejmanek et al. 2005a). The success of IAS is often attributed to their capacity for rapid growth through high resource acquisition, particularly in non-resource limited conditions (Pyšek and Richardson 2008; Funk 2013). However, Leishman et al. (2010) showed that IAS and native species do not have fundamentally different carbon capture strategies, supporting the concept of community invasibility, i.e. characteristics of the resident communities favouring invasion (Richardson and Pyšek 2006). Specifically, according to trait-environment relationships (Dalle Fratte et al. 2019), differences between IAS and native plant species can reflect environmental conditions of the sites where they occur rather than differences between species *per se* (Leishman et al. 2010).

Pyšek and Richardson (2008) reviewed the literature in an attempt to identify the traits characteristic of invasive vascular plant species, finding evidence only for some traits that are universally associated with these species: height, vigorous vegetative growth, early and extended flowering. Van Kleunen et al. (2010) demonstrated that IAS tend to outcompete native or non-invasive species due to higher values of functional traits related to growth rate and resource acquisition. Also consistent with a ‘soft leaves, fast growth’ strategy, the most successful alien species are those that minimize carbon investment in leaf construction, as observed both in terrestrial (Tordoni et al. 2019) and aquatic vascular flora (Lukacs et al. 2017). Nevertheless, the debate concerning native vs. alien suites of traits is ongoing since patterns or common features are not always clear (Hulme and Bernard-Verdier 2018; Funk et al. 2017). Daehler (2003) suggested that there is no unique set of traits responsible for invasiveness, but that multiple suites of traits could explain invasion success in different environments (e.g. Tecco et al. 2010; Funk et al. 2016).

Multiple adaptive traits can be summarized in terms of the ecological strategies or the group of functional traits involved in plant resource economics (determining matter and energy turnover rates) and size (denoting the absolute amount of matter and energy present within organisms and within biotic communities) (Diaz et al. 2016; Pierce et al. 2017). Grime's CSR life-strategy theory provides a conceptual framework to classify species into competitive (C), stress-tolerant (S) and ruderal (R) strategies (Grime and Pierce 2012), and is currently the only plant strategy theory that agrees with resource economics and size as the principal axes of adaptive variation, and can place these in the context of environmental selection pressures (Pierce and Cerabolini 2018). There are a range of advantages in using adaptive strategy theory, rather than single traits, to evaluate the interactions among alien and native species (Davis 2009; Rejmanek et al. 2005a; Guo et al. 2018, 2019) and the effects on ecosystem services provision due to plant invasion (Vicente et al., 2013). The application of CSR strategy theory to studies at a local to regional-scale, has highlighted that amongst alien plant species, R- and C- selected species and their intermediate strategies (CR) are prevalent, while S-selected species are under-represented (Pyšek et al. 2003; Lambdon et al. 2008a; Dainese and Bragazza 2012; Alexander et al. 2016). Despite this pattern also being evident at the global scale (Guo et al. 2018, 2019), Hulme and Bernard-Verdier (2018) raised some questions concerning the use of CSR theory to categorize alien species, since it may mask individual trait differences. Indeed, though CSR strategies can potentially provide a robust theoretical context to predict the performance of species in a definite environment (Grime and Pierce 2012), it is necessary to experimentally validate the potential for CSR strategy theory to discriminate IAS and to predict their capacity to invade.

In this study, we used a large regional flora dataset based on IAS and native species characteristic of Southern European vegetation to test two hypotheses. 1) First we tested whether IAS and native plant species occupy the same or a different space within the ternary combination imposed by CSR strategies; hence, contrary to the habitat invasibility concept (Leishman et al. 2010), the capacity to invade depends on differences in carbon accumulation strategies. 2) A secondary hypothesis was that at the regional scale the invasion success of alien species is linked to a higher degree of C- and R-selection, according to recent global findings of naturalization success of alien species (Guo et al. 2018). We tested these hypotheses for different growth forms (trees, shrubs, long-lived herbs, short-lived herbs, hydrophytes) using plant functional traits common across growth forms (Pierce et al. 2017).

2. Methods

2.1 Study area

The study area was the Lombardy administrative region of Italy, which covers an area of 23,870 km2 ranging between latitudes 44°40' – 46°37' N, from the Apennines to the Alps, and longitudes 8°29' – 11°25' E, from Lake Maggiore to Lake Garda. The elevation spans 10m a.s.l. in the Po Valley to approximately 4,000m a.s.l. on Mount Bernina. The study area includes both the Alpine and Continental biogeographical regions (ETC/BD 2006), and spans from continental to oceanic bioclimates (Pesaresi et al. 2014), although it is characterized by a highly variable mesoclimate due to its complex orography. Geological substrates consist of a wide range of litho-types, both silicate or carbonate rocks, alluvial or morainic deposits. This extensive environmental variability is consistent with the heterogeneity of vegetation communities, ranging from Mediterranean holly oak woods near Lake Garda to Alpine tundra in the highest mountain regions.

2.2 Dataset

The dataset included all records of plant functional traits for the species analysed by Dalle Fratte et al. (2019), to which we added some unpublished data. Many records are available from Authors' datasets in TRY (Kattge et al. 2011; https://www.try-db.org/ see datasets n. 227, 228, 229, 371, 372 and related references).

IAS are defined as naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants (approximate scales:>100m /< 50 years for taxa spreading by seeds and other propagules; > 6m / 3 years for taxa spreading by roots, rhizomes, stolons, or creeping stems), and thus have the potential to spread over a considerable area (Richardson et al. 2000). We established the status of native or invasive species in Lombardy according to literature (Assini et al. 2010; Galasso et al. 2018).

A rapid decrease in alien species richness with increase in elevation was detected in adjacent regions of Italy (Barni et al. 2012) and more generally in the European Alps (Becker et al. 2005). The upper montane belt was considered the highest elevation attained by alien species, beyond which they were rarely observed. Given the high elevation gradient of the study area, species exclusively growing in subalpine or alpine belts, i.e. with the ecological index of T (temperature) from 1 to 2.5 according to Landolt et al. (2010), were removed from the dataset. In the analysis, we also considered species listed as "x" for T index (Landolt et al. 2010), since they can spread along a wide portion of the elevation gradient.

The final dataset thus consisted of 1039 records, including 59 tree species, 94 shrubs, 601 long-lived herbs (perennial graminoids and forbs), 224 short-lived herbs (annual or biennial graminoids and forbs) and 61 hydrophytes (Table 1). We assigned each species to the growth forms identified by Guo et al. (2018): trees, shrubs, long-lived herbs, and short-lived herbs, but considered hydrophytes separately, given their specific ecological constraints. Finally, we computed the C-, S-, and R- scores for each species according to the *StrateFy* tool of Pierce et al. (2017).

Table 1: Summary of the number (n) and percentage of invasive alien and native species within all the dataset and each growth form.

	n	%
ALL SPECIES	**1039**	**100.0**
invasive	110	10.6
native	929	89.4
TREES	**59**	**100.0**
invasive	17	28.8
native	42	71.2
SHRUBS	**94**	**100.0**
invasive	16	17.0
native	78	83.0
LONG-LIVED HERBS	**601**	**100.0**
invasive	22	3.7
native	579	96.3
SHORT-LIVED HERBS	**224**	**100.0**
invasive	46	20.5
native	178	79.5
HYDROPHYTES	**61**	**100.0**
invasive	9	14.8
native	52	85.2

2.3 Phylogenetic tree

We built a phylogenetic tree of the species in our dataset using the *PhytoPhylo* megaphylogeny tree implemented by Qian and Jin (2016). Following their recommendations, we used their ‘Scenario 1′ to solve the phylogeny at the species level in our dataset. This scenario is the most cautious, as it adds genera or species as basal polytomies within their families or genera to avoid random solutions. Before processing the phylogenetic tree, names of every taxon (family, genus and species) in our dataset were standardized according to The Plant List (www. theplantlist.org) using the R package ‘*Taxonstand*’ (Cayuela et al. 2017). *2.4. Statistical analysis* The CSR classification indicates proportion for each element (C, S and R) along each axis, and thus represents an integrated trade-off between traits, i.e. the three axes are dependent on each other (Pierce et al. 2017). We used the R package ‘*compositions*’ (van den Boogaart et al. 2018) to visualize the ternary diagrams of the CSR strategies of IAS and native species. To account for the compositional structure of the data (van den Boogaart and Tolosana-Delgado 2013) we first transformed the dataset of CSR scores with Isometric

Log-Ratio Transformation (ilr), which reduced the ternary dataset to a two dimensional image. To test the first hypothesis, i.e. whether invasive and native plant species occupy different CSR space, we computed phylogenetic multivariate analysis of variance (MANOVA) which compares native vs. invasive alien species inside the distribution provided by the ternary combination of CSR scores. In addition, to check for the second hypothesis, i.e. whether the invasion success of alien species is correlated with a higher degree of C- and R- selection, we computed a phylogenetic univariate analysis of variance (ANOVA) considering each axis (C-, S-, and R-) as independent. The *StrateFy* CSR classification method (Pierce et al. 2017) does not use each trait (leaf area, LA; leaf dry matter content, LDMC; specific leaf area, SLA) to directly represent the extent of C-, S- and R- selection, rather they are calculated using the trade-offs between traits, integrated and compared against trade-offs evident globally. Hence, to gain an immediate comparison with previous traits-based studies on IAS, we also ran a phylogenetic ANOVA on each leaf trait used for the calculation of CSR strategies (LA, LDMC and SLA). Before running the ANOVAs, we checked for normality of C-, Sand R- scores and leaf traits by means of the Shapiro-Wilk normality test. While it was not necessary to transform the C-, S- and R- scores, the transformation that best normalized the leaf traits were *log(x+1)* for LA and SLA, and *sqrt(x)* for LDMC. We computed both phylogenetic MANOVA and ANOVA by means of the function 'aov.phylo' in the R package '*Geiger*' (Harmon et al. 2007), which accounts for the phylogenetic tree produced by the '*S.PhyloMaker*' R function (Qian and Jin, 2016). We used the R package 'ggplot2' (Wickham 2016) to visualize the boxplots of the C-, S- and R- scores and then of leaf traits between IAS and native species. We ran both the analysis considering all the species together, as well as splitting the dataset according to the growth forms. We performed all the analysis with the software R 3.4.4 (R Core Team 2018).

3. Results

In the CSR ternary space and accounting for the phylogeny (Fig. 1 and Table 2), IAS did not show any significant divergence from native species regardless of the growth form. Considering growth forms, IAS were significantly different from native species only within trees (p=0.02). Invasive alien trees showed higher C- and lower S- compared to natives, but similar R- mean values (CSR compositional mean ± SD: 51.2 (C) : 38.0 (S) : 10.8 (R) ± 24.7% vs 40.2 (C) : 46.3 (S) : 13.5 (R) ± 22.5%) (Appendix A). All gymnosperm trees were classified as stress tolerant, indeed, they were clustered in the S corner of the CSR triangle; however, this did not affect the results since in our dataset gymnosperm trees were balanced between IAS and native species. Shrubs, long-lived herbs, short-lived herbs and hydrophytes did not differ between IAS and native species.

Analysing axes of CSR as independent variables and considering the phylogenetic signal (Fig. 2 and Table 3), at all species level we found higher C- scores for IAS compared to natives (mean ± SD: 36.3 ± 22.6% vs 29.8 ± 21.4%), without significant differences between S- and R- scores. Trees showed the same pattern, with IAS showing higher C- scores (44.5 ± 19.4% vs 34.6 ± 13.7%) (Appendix B). Also shrub IAS showed a marked increase of C- scores compared to native species (43.5 ± 13.8% vs 23.9 ± 17.0%), but they showed significantly lower S- scores too (35.9 ± 18.0% vs 54.8 ± 26.0%). Among herbaceous growth forms, invasive alien short-lived herbs had higher C- scores than native species (35.5 ± 21.5% vs 26.3 ± 22.8%), while S- and R- scores did not show significant differences. Among longlived herbs and hydrophytes, IAS did not show significant differences from native species along each CSR axis. With regard to the single leaf traits, and accounting for the phylogenetic signal (Fig. 3 and Table 4), at the species level we found significant differences only for LA, being larger for IAS compared to natives (mean ± SD: 7.4 ± 2.2 vs. 6.6 ± 1.9) (Appendix C). A similar pattern was observed among shrubs (mean ± SD: 8.4 ± 1.0 vs. 6.4 ± 1.9) and short-lived herbs (mean ± SD: 7.1 ± 1.6 vs 6.0 ± 2.0), but for the other growth forms IAS and native species did not display any significant difference for each considered leaf trait.

Table 2: Results of the phylogenetic multivariate analysis of variance (MANOVA) among invasive alien and native species CSR compositional data within all species and each growth form, indicating Wilk's statistics, F-values and phylogenetic p-values. Coefficients in bold are statistically significant ($p < 0.05$).

	Wilks	F	p-value
all species	0.998	0.92	0.60
trees	**0.862**	**4.50**	**0.02**
shrubs	0.960	1.87	0.25
long-lived herbs	0.999	0.37	0.85
short-lived herbs	0.978	2.46	0.27
hydrophytes	0.935	2.01	0.38

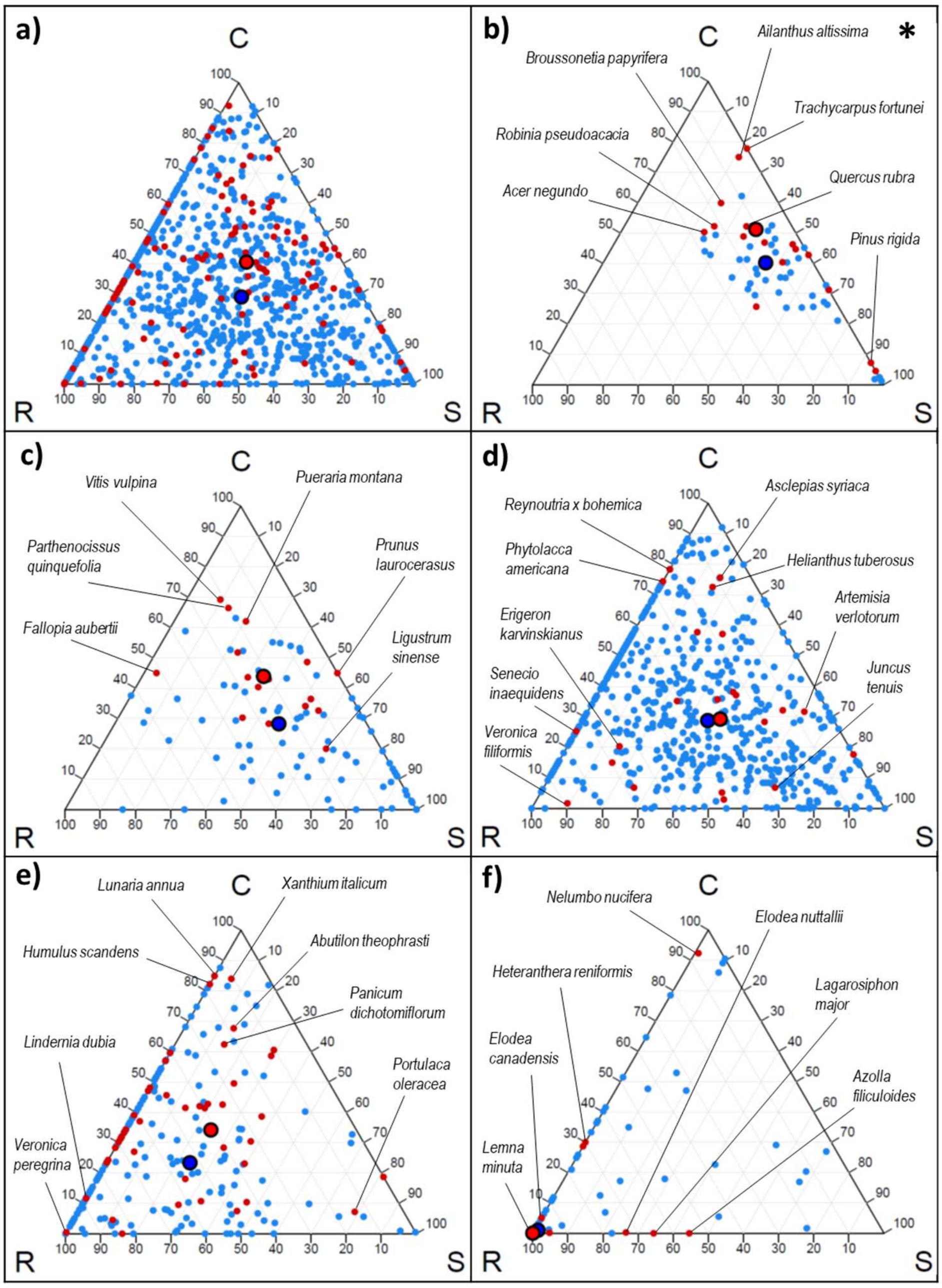

Figure 1: Invasive alien (red dots) and native (blue dots) species within the CSR strategies triangle. Legend: a) all species, b) trees, c) shrubs, d) long-lived herbs, e) short-lived herbs, f) hydrophytes. Star indicates statistically significant difference between invasive and native species ($p < 0.05$).

Table 3: Results of the phylogenetic univariate analysis of variance (ANOVA) among invasive alien and native species C-, S-, and R- scores, within all species and each growth form, indicating F-values and phylogenetic p-values. Coefficients in bold are statistically significant (p<0.05).

	C		**S**		**R**	
	F	**p-value**	**F**	**p-value**	**F**	**p-value**
all species	**9.09**	**0.03**	1.76	0.27	1.17	0.47
trees	**4.91**	**0.05**	1.68	0.29	0.88	0.50
shrubs	**18.70**	**0.01**	**7.68**	**0.02**	0.02	0.89
long-lived herbs	0.26	0.66	0.01	0.96	0.38	0.69
short-lived herbs	**6.08**	**0.05**	0.00	0.98	5.01	0.15
hydrophytes	0.25	0.82	0.00	0.97	0.14	0.83

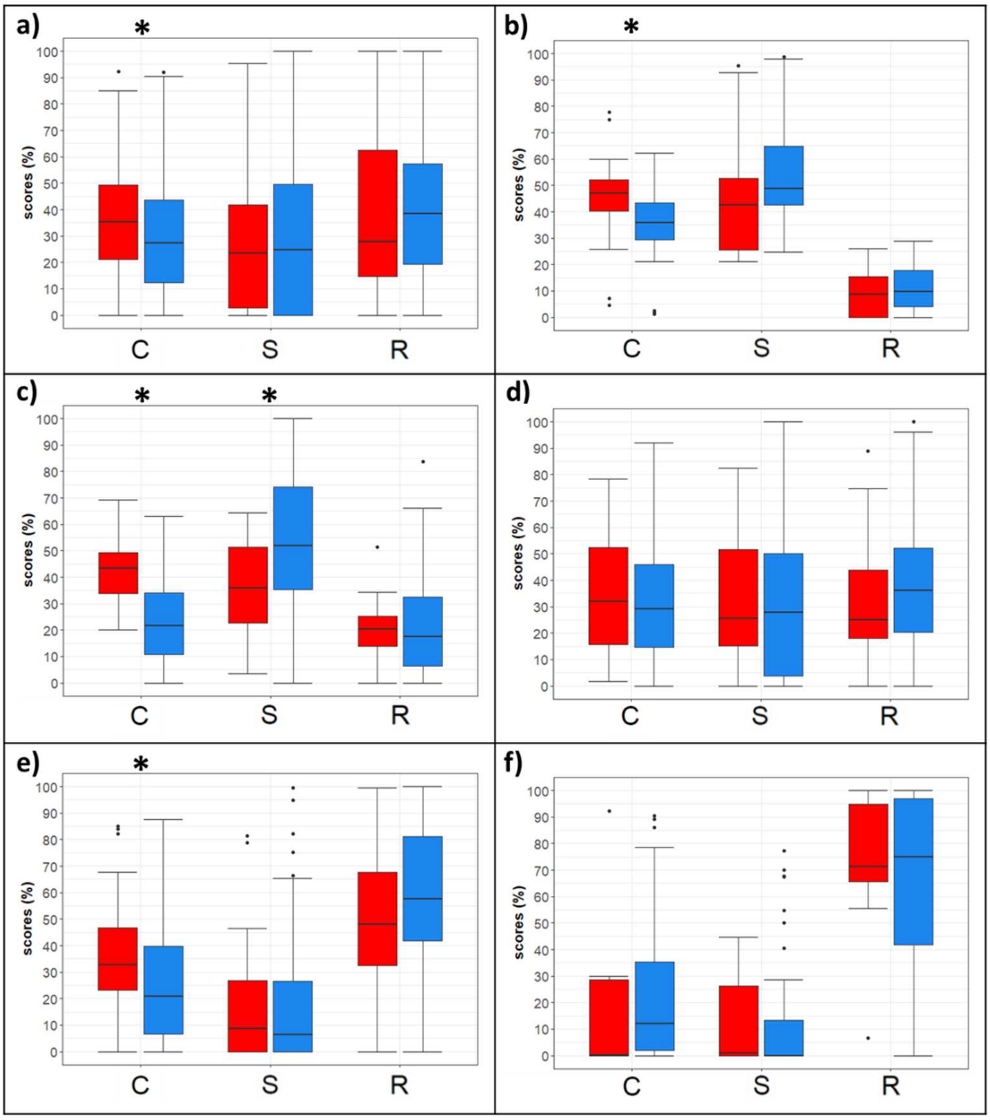

Figure 2: Boxplots of C-, S-, R- strategy scores of invasive alien (red) and native (blue) species displaying the median (line in the middle of the boxes), the interquartile range (boxes), ± 1.5 times the interquartile range (whiskers) and outliers (circles) for all species and each growth form. Legend: a) all species, b) trees, c) shrubs, d) long-lived herbs, e) short-lived herbs, f) hydrophytes. Star indicates statistically significant difference between invasive alien and native species (p<0.05).

Table 4: Results of the phylogenetic univariate analysis of variance (ANOVA) among invasive alien and native species leaf traits (leaf area, LA; leaf dry matter content, LDMC; specific leaf area, SLA), within all species and each growth form, indicating F-values and phylogenetic p-values. Coefficients in bold are statistically significant (p<0.01).

	LA		**LDMC**		**SLA**	
	F	**p-value**	**F**	**p-value**	**F**	**p-value**
all species	**14.44**	**0.01**	2.43	0.14	1.05	0.42
trees	4.77	0.08	0.16	0.73	0.32	0.64
shrubs	**17.63**	**0.01**	0.54	0.52	4.53	0.06
long-lived herbs	0.57	0.61	3.24	0.24	0.20	0.74
short-lived herbs	**12.02**	**0.01**	0.10	0.77	0.49	0.62
hydrophytes	1.10	0.41	1.92	0.36	1.79	0.36

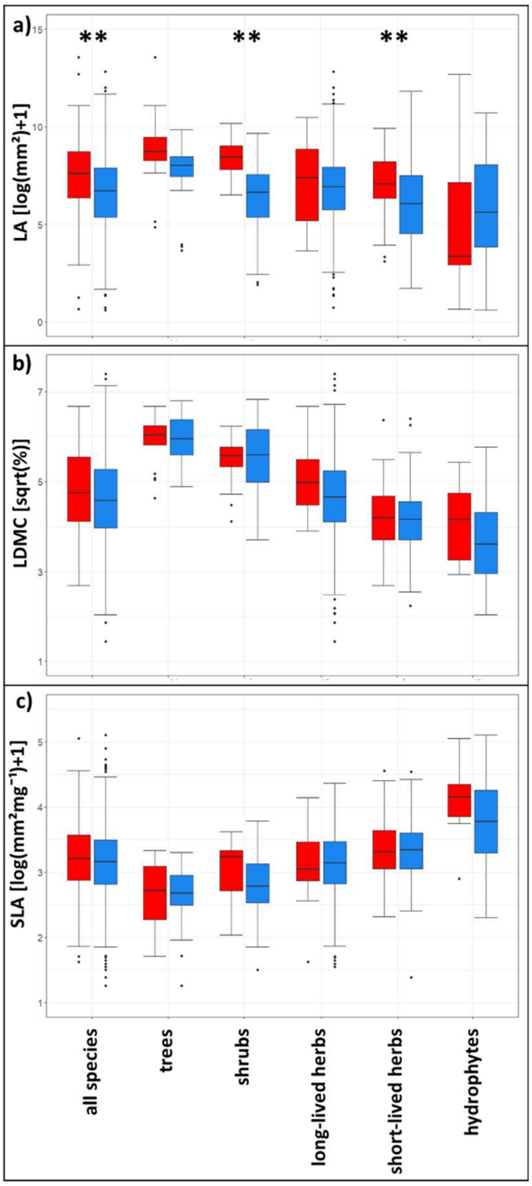

Figure 3: Boxplots of a) leaf area (LA), b) leaf dry matter content (LDMC), and c) specific leaf area (SLA) of invasive alien (red) and native (blue) species displaying the median (line in the middle of the boxes), the interquartile range (boxes), ± 1.5 times the interquartile range (whiskers) and outliers (circles) for all species and each growth form. Star indicates statistically significant difference between invasive alien and native species (p<0.01).

4. Discussion

Our results, based on a large regional dataset of plant species characterizing the vegetation of contrasting biogeographic situations of Southern Europe, underline the relevance of CSR strategy theory (Grime and Pierce 2012) to assess the invasiveness ability of IAS. We can confirm that there are clear patterns of CSR strategies at the regional scale (Pyšek et al. 2003; Lambdon et al. 2008a; Dainese and Bragazza 2012; Alexander et al. 2016), which confirm and emphasize recent observation at the global scale concerning species naturalization incidence and extent based on similar approach (Guo et al. 2018). Nevertheless, we observed some different responses among growth forms and with regard to the CSR space.

Our first hypothesis was that IAS and native plant species occupy a different region of CSR space. We detected clear differences only for trees, while robust patterns were not evident when all growth forms were considered together, or separately, for non-tree growth forms (Fig. 1 and Table 2). In other words, except for trees, the space occupied by IAS and native species in the CSR classification was practically identical. This finding suggests that IAS occupy a similar strategic position to native species and that other factors operate in concert with carbon accumulation strategies to drive the local success of invasion (Funk et al. 2017). For example, these factors could be linked to the phylogenetic similarity of alien and native species, which involves that IAS prefer to occupy existing phylogenetic space in native communities (Ordonez 2014; Loiola et al. 2018). Furthermore, native communities can determine the entry of IAS depending by intrinsic habitat properties such as habitat filtering (Carboni et al. 2016) and invasibility (Leishman et al. 2010; Richardson and Pyšek 2006; Rejmanek et al. 2005b), or to biogeographical context (Pyšek and Richardson 2006). Hence, with the exception of trees, when different environmental gaps are available for colonization within a plant community, IAS and native species should have the same probability to colonize them. Such findings are consistent with difficulties encountered in the management of IAS, as they respond to multiple components of global change (Bradley et al. 2010) and do not differ solely with regard to plant functional types or strategies.

Consequently, IAS management must be integrated into the framework of whole vegetation management, since it is context-dependent (Daehler 2003) and may differ across communities (Funk et al. 2017). The markedly different patterns between trees and the other growth forms within the CSR space (Fig. 1), underline that regional efforts to control plant invasion should focus on tree IAS as a priority. Indeed, while non-tree IAS confirm the 'join-the locals' hypothesis (Thompson et al. 1995), according to which the filtering of environmental factors led to strong similarities between IAS and natives (e.g., Tecco et al. 2010), patterns among trees indicated divergence, following the 'tryharder' hypothesis (Crawley et al. 1996). This latter hypothesis maintains that successful aliens should be able to deal better with the local conditions than resident species. Specifically, tree IAS were more competitive compared to natives in resource-rich habitats (favouring the C- selection strategy), in line with observations for woody species by Tecco et al. (2010). The 'try-harder' hypothesis should work symmetrically on the other side of the nutrient gradient, i.e. towards resource-poor habitats, where IAS possessing attributes associated with resource conservation and slow growth should succeed over native species with similar strategies (Funk and Vitousek 2007). According to CSR classification, both IAS and native coniferous species are classified at the most conservative extreme of the S- selection gradient, but due to the few species data available we unfortunately could not provide a statistically confirmation of a trend.

The second hypothesis was that the invasion success of IAS at the regional scale is associated with a high degree of C- and R- selection strategies, according to recent global findings of naturalization success of alien species (Guo et al. 2018). Analysing each axis of CSR as independent variables, we found broad evidence in support of the hypothesis that IAS tend to be competitors (Fig. 2). Efficient competitors for available resources are likely to be the best invaders in natural and semi-natural ecosystems (Pyšek et al. 1995; Rejmanek et al. 2005a).

Moreover, our findings underline that the evident selection of IAS toward a higher competitive ability is reflected in larger leaves of IAS compared to native species (Fig. 3), LA being the only trait showing significant differences. This pattern is significant when considering all species together, as well as among trees (not for LA), shrubs and shortlived herbs, but not among long-lived herbs and hydrophytes (Tables 3 and 4). Competitors are characterized by traits that enhance photosynthetic rate and rapid growth, larger leaves, high flowering frequency and nutrient rich leaves (Wright et al. 2004; Reich 2014) and some of them (the most competitive) have been associated also to a high efficiency of clonality (Song et al. 2013). All these traits have been frequently linked with invasive plant species (Pyšek and Richardson 2008; van Kleunen et al. 2010; Roiloa et al. 2016). While very few alien species are able to invade plant communities at advanced successional stages (Rejmanek et al. 2005a), IAS tend to accumulate in early successional stages (Crawley et al. 1996), i.e. following disturbance or as part of secondary succession. In particular, early succession on fertile substrates typically promotes dominance of high-resource demanding species (Grime 2006). Indeed, high-resource ecosystems tend to accumulate more exotic species than low-resource ecosystems (e.g. Huenneke et al. 1990; Gross et al. 2005; Stohlgren et al. 2008). After that IAS are released into new environments, usually because they naturalize from cultivated gardens (Guo et al. 2019), such sites may be the gateway through which alien species invade a target region (Crawley et al. 1996; Rejmanek and Richardson 1996; Pyšek et al. 2017).

Despite the fact that Guo et al. (2018) found a positive relation of Rselection with the incidence of naturalization worldwide, we did not find such evidence for IAS at the regional scale in our study system. It is possible that such differences are caused by the more specific subset of species that we used compared to the global dataset. Indeed, our analysis might be considered a specific focus of part of the study by Guo et al. (2018), as IAS are the subset of naturalized species already having extensive impact on the structure of communities and ecosystems (Pejchar and Mooney 2009). Moreover, focusing on IAS we removed the naturalized species from our analyses, which constitutes a large proportion of the local flora (e.g., Galasso et al. 2018) that is more likely to contain a high degree of ruderals. Often such invasions start from disturbed sites (Crawley et al. 1996; Richardson et al. 2000; Pyšek et al. 2017), where species with a ruderal life strategy thrive (Rejmanek and Richardson 1996). Hence, IAS act on ecosystem that are already modified, characterized by a lower disturbance. Furthermore, a global analysis such as that of Guo et al. (2018) represents a greater range of very specialized environments. For example, they highlighted that the high R- degree of shrubs and long-lived herbs was due to the presence of Mediterranean species, which are absent in our local flora. In addition, Guo et al. (2018) studied the naturalization capacity of species worldwide, and it is possible that some of the species we classified as native in our geographic region are classified as naturalized elsewhere in the world.

With regard to the degree of S- selection, we did not find differences either within or between growth forms. Only invasive alien shrubs displayed significant lower values of S-scores compared to natives, confirming that species with a greater S-score are less likely to become naturalized (Pyšek et al. 2003; Alexander et al. 2016). Usually stresstolerators are very long-lived, have a long leaf life-span, grow slowly, fruit later and produce fewer seeds with respect to competitors and ruderals (Grime 1979; Grime and Pierce 2012). All of these traits contrast with those commonly reported to be associated with successful invaders (Pyšek and Richardson 2008; van Kleunen et al. 2010), and could make stress-tolerators less likely to be selected by humans as horticultural or gardening plants (van Kleunen et al. 2018; Guo et al. 2019). In general, stress-tolerant plants rely heavily on vegetative rather than sexual reproduction and rarely show extensive morphological plasticity, which limits their potential to spread (Grime 1979, 1988). In addition, the chance of a stress-tolerant plant to be introduced into an environment with a stress regime comparable to the one it is adapted to in its native range is relatively low, given that stressful environments are relatively rare in general, and often characterised by idiosyncratic and restricted environmental regimes (Alexander et al. 2016; Funk 2013). Native species appear to have a competitive advantage over IAS in low-resource ecosystems (Alpert et al. 2000; Daehler 2003), and communities become more susceptible to invasion when resource availability is increased (Davis et al. 2000; Funk 2013).

According to Grime (2001), woody species occupy the S-C region of the CSR triangle, while trees with high R-scores do not exist (Pierce et al. 2013, 2017). In the S- corner we found a cluster of gymnosperm tree species, while broad-leaved trees were classified towards higher C (see also Pierce et al. 2013). Two IAS, i.e. *Ailanthus altissima* and *Trachycarpus fortunei*, exhibited the highest C-scores. In general, among IAS we found a solid signal towards more competitive species, considering both the CSR ternary combination, and the dimension of each single axis. Indeed, above all growth forms, trees were the only one to show significant differences within the CSR space. For trees, it is likely that IAS are fast-growing species that can more rapidly fill empty gaps following clear-cuts, while late-successional species have not been imported, except for a few exceptions (e.g. *Quercus rubra*). Guo et al. (2018) found that the naturalization incidence and extent of trees is not, or is only weakly, affected by their C-, S-, and R- scores. Differences with our findings could be due, once again, to the fact that we have considered IAS, i.e. the portion of naturalized species that are already well established in new environments. Indeed, the naturalization of trees may occur over longer periods of establishment, long generation time, relatively low proportions of resources invested in seed production (Grime 1979), and the long time lag following introduction to new ranges (Kowarik 1995; Pyšek et al. 2017). Shrubs also prevail in the SC region of the triangle (Grime 2006; Pierce et al. 2013). However, in this case we found robust patterns only considering each axis as independent. The highest C-scores were associated with climber IAS such as *Vitis vulpina* and *Parthenocissus quinquefolia* as well as to *Pueraria montana*; the latter is a clonal species, which highlights that high clonality can correspond to greater invasive capacity (Song et al. 2013; Roiloa et al. 2016). In our dataset, the shrubs with the highest R- score were native species, i.e. *Thymus vulgaris* and *Myricaria germanica* (Fig. 1).

With regard to herbaceous growth forms, short-lived IAS showed high C-scores compared to natives according to Guo et al. (2018). Accordingly, short-lived herbs do not exhibit extensive ruderalism, probably because they are successfully invaders only under conditions of relatively low disturbance, while under continuous disturbance, the seed production of ruderal species does not compensate for the mortality rate, and they eventually fail to establish (Grime 1979, 1988). Regarding long-lived herbs, it must be considered that in our dataset IAS represent only 3.7% of the total (Table 1), which may have affected outcomes; anyway, invasive alien long-lived herbs are homogenously distributed in the CSR space (Fig. 1), without forming clusters, similarly to natives. However, competitive and large-leaved invasive herbs capable of forming monospecific stands in productive habitats were classified towards the C-selected corner, for example *Phytolacca americana*, *Reynoutria* x *bohemica*, or *Helianthus tuberosus* (Pierce et al. 2013). Aquatic plants displayed clear preferences towards ruderal strategies, for both IAS and native species (Fig. 1), confirming previous observations (Pierce et al. 2012, 2013). Aquatic species were either small, fast growing and with extremely acquisitive leaves (highly R-selected) or CS to C-selected species with large, broad leaves, such as water lilies, in agreement with previous conclusions (Pierce et al. 2012). Among IAS, *Nelumbo nucifera* displayed the highest C-scores, and in the CSR spectrum clustered together with other native *Nymphaeiden* (Pierce et al. 2012). Several traits of this species may form part of the C- selected syndrome (Bornette et al. 2008), including moderate relative growth rates, limited vegetative dispersal and seeds that sink immediately. Many aquatic species are R selected in the extreme, as they mainly rely on rapid generation to face disturbance, indeed most hydrophytes are typical of disturbed habitats, as they colonize areas where seasonal flooding washes away the existing vegetation (Bornette et al. 2008). No hydrophyte species in our study exhibited selection toward the extreme of conservative S- corner, also confirming that hydrophyte IAS may not include stress tolerators *sensu* Grime (1979) (Kautsky 1988; Pierce et al. 2012). Previous observations of functional traits of hydrophytes (Lukacs et al. 2017) found that traits of IAS are linked to competitive ability ('soft leaves faster'). However, we cannot confirm such differences because the functional traits of alien aquatic plants depend strongly on their growth-form (see Lukacs et al. 2017).

5. Conclusions

In the ternary space determined by CSR classification, IAS and native species cover almost the same region, indicating that IAS can occupy the same niches and have the same requirements of native species. This supports the idea that a complex of factors, which act in conjunction with the carbon accumulation strategies, mediates the mechanisms that regulate the invasion of empty gaps in vegetation communities. Several authors stressed that the spread of IAS is linked to the pivotal contribution of: 1) the phylogenetic similarity between alien and native species (Ordonez 2014; Loiola et al. 2018); 2) the capability of habitat features to drive IAS establishment (Carboni et al. 2016); 3) habitat invasibility (Richardson and Pyšek 2006; Leishman et al. 2010; Rejmanek et al., 2005b), 4) as well as the role of biogeographical context (Pyšek and Richardson 2006). Analysing each axis of CSR independently, as well as separate leaf traits (LA, LDMC and SLA), we found broad evidence that IAS are mainly true competitors, suggesting that traits that can describe the invasiveness ability of alien plants at the regional scale are those underlined by the C-selection strategy. Moreover, contrary to Hulme and Bernard-Verdier (2018), the CSR scores, being calculated using the trade-off between traits, provide more robust differences between IAS and native species compared to individual trait variation.

Further studies are necessary to implement our findings; in particular, we need to understand differences among distinct environments and habitats according to Grime's theory and the ecology of invasive plants (Rejmanek et al. 2005b; van Kleunen et al. 2018). Global changes and the flora globalization will promote the establishment of competitive alien functional types in Southern Europe (Walther et al. 2002; Ciappetta et al. 2016; Najberek et al. 2017; Dalle Fratte et al. 2019). In this context, CSR theory can be a viable tool for the risk assessment of IAS (Vicente et al., 2013), implementing the current approaches of prioritization methods and horizon scanning (Carboneras et al. 2018), and the development of non-invasive ideotypes of crops and horticultural plants (Anderson et al. 2006; van Kleunen et al. 2018; Guo et al. 2019).

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7. Supplementary material

Appendix A: CSR compositional mean (mean ± standard deviations) within all species and the analysed growth forms.

		C : S : R ± sd
ALL SPECIES	**invasive**	40.4:32.0:27.5 ± 25.1
	native	28.9:36.3:34.8 ± 25.8
TREES	**invasive**	51.2:38.0:10.8 ± 24.7
	native	40.2:46.3:13.5 ± 22.5
SHRUBS	**invasive**	43.9:34.6:21.6 ± 17.4
	native	28.2:46.7:25.0 ± 26.0
LONG-LIVED HERBS	**invasive**	40.4:32.0:27.5 ± 23.9
	native	29.0:36.6:34.4 ± 25.1
SHORT-LIVED HERBS	**invasive**	34.2:24.3:41.5 ± 25.6
	native	23.4:23.8:52.9 ± 29.2
HYDROPHYTES	**invasive**	0.1:0.0:99.9 ± 36.7
	native	0.3:0.0:99.7 ± 35.6

Appendix B: C-, S-, and R-scores (mean ± standard deviations) within all species and the analysed growth forms.

		C (%) ± sd	S (%) ± sd	R (%) ± sd
ALL SPECIES	**invasive**	36.3 ± 21.4	26.4 ± 27.8	37.3 ± 26.3
	native	29.8 ± 21.6	30.0 ± 27.4	40.2 ± 26.5
TREES	**invasive**	44.5 ± 19.4	46.4 ± 22.8	9.1 ± 9.0
	native	34.6 ± 13.7	53.9 ± 19.1	11.5 ± 8.7
SHRUBS	**invasive**	43.5 ± 13.8	35.9 ± 18.0	20.6 ± 11.9
	native	23.9 ± 17.0	54.8 ± 26.0	21.3 ± 19.4
LONG-LIVED HERBS	**invasive**	34.4 ± 25.1	31.6 ± 23.3	34.0 ± 24.7
	native	32.0 ± 21.2	31.0 ± 27.2	37.0 ± 22.1
SHORT-LIVED HERBS	**invasive**	35.5 ± 21.5	15.9 ± 20.0	48.6 ± 24.2
	native	26.3 ± 22.8	15.7 ± 20.8	58.0 ± 25.5
HYDROPHYTES	**invasive**	17.4 ± 30.7	12.4 ± 17.8	70.3 ± 28.3
	native	22.1 ± 25.3	11.9 ± 21.2	65.9 ± 32.9

Appendix C: Values of leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA) (mean ± standard deviations) within all species and the analysed growth forms. Values refer to transformed data: *log(x+1)* for LA and SLA, *sqrt(x)* for LDMC.

		LA ± sd	**LDMC ± sd**	**SLA ± sd**
ALL SPECIES	**invasive**	7.4 ± 2.2	4.8 ± 1.0	3.2 ± 0.6
	native	6.6 ± 1.9	4.6 ± 1.0	3.2 ± 0.5
TREES	**invasive**	8.7 ± 2.0	5.9 ± 0.6	2.6 ± 0.5
	native	7.7 ± 1.4	6.0 ± 0.5	2.7 ± 0.4
SHRUBS	**invasive**	8.4 ± 1.0	5.4 ± 0.6	3.1 ± 0.4
	native	6.4 ± 1.9	5.6 ± 0.8	2.8 ± 0.5
LONG-LIVED HERBS	**invasive**	7.1 ± 2.1	5.0 ± 0.7	3.1 ± 0.5
	native	6.8 ± 1.8	4.6 ± 0.9	3.1 ± 0.5
SHORT-LIVED HERBS	**invasive**	7.1 ± 1.6	4.2 ± 0.8	3.4 ± 0.5
	native	6.0 ± 2.0	4.2 ± 0.6	3.3 ± 0.4
HYDROPHYTES	**invasive**	4.8 ± 3.7	4.1 ± 0.9	4.1 ± 0.6
	native	5.8 ± 2.4	3.6 ± 0.9	3.8 ± 0.7

Soil–plant interactions in a pasture of the Italian Alps

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Journal of Plant Interactions, 15:1, 39-49,
https://doi.org/10.1080/17429145.2020.1738570

Abstract

A detailed assessment of a pasture's functioning based on soil properties characterization, floristic composition, and 'functional summary' by evaluating competitor–stress tolerator–ruderal (CSR) strategies is provided for a doline in Central Italian Alps. A floristic survey was carried out at 35 sampling points, representative of the main topographic features, soil and vegetation types; the functional profile at the community level was evaluated by assessing for each species its Grime's CSR strategy; each point was characterized through soil profiles and topsoil (0–10 cm) sampling; pH, soil organic carbon and total nitrogen, available P, soil humus fraction, root density, bulk density, water content, and available water capacity were determined. Our study showed i) a strong relationship between vegetation, soil properties, topography, and grazing; ii) a prevalence of stress-tolerant strategies; iii) the ability of plant strategy variation to reflect the ecological parameters; and iv) the vegetation potentiality to be an indicator of environmental spatial variability.

Keywords Alpine pasture, Spatial variability, Soil diversity, Plant community, CSR strategy.

1. Introduction

Natural alpine environments are often characterized by great spatial variability in their geomorphological, geological, pedological, and vegetation properties. This variability sometimes occurs at short spatial scales (Aalto et al. 2013) and is strongly conditioned by highly dynamic geomorphic processes.

In alpine ecosystems, soil–plant relationships involve a wide range of essential environmental issues by affecting ecosystem biodiversity, soil potentiality as a carbon sink, and biogeochemical processes, while also representing a valid indicator of the global warming response (Qin et al. 2007; Grand et al. 2016).

The relationships between soil and vegetation in alpine environments have long been studied (Isard 1986; Rose et al. 1988; Gensac 1990; Darmody et al. 2004; Lane et al. 2016). Some studies showed the importance of edaphic factors by investigating physical parameters (texture, soil thickness, surface characteristics; Rubio and Escudero 2000), chemical parameters (pH, but also soil fertility, in particular N and P; Anic et al. 2010; Ahmad et al. 2016), or water availability (Kammer et al. 2013). Other factors, such as climate (Zelnik and Čarni 2013), bedrock (Toure et al. 2015), and topographic aspects (absolute and relative altitude, slope, etc.; Zhang and Hu Gang 2013), have been investigated to explain vegetation variability and characteristics. Plant cover variability is also affected by management (chemical or organic fertilization, livestock use and grazing intensity (Marini et al. 2008; Teuber et al. 2013). In some cases it has been shown that the soil–vegetation relationship may be expressed in a synthetic way simply using the pedological taxonomy (Gensac 1990; Caria et al. 2015; Grand et al. 2016).

Ecosystem properties are reflected by variation in the adaptive traits of plant species, which may reflect the main ecological gradients shaping plant phenotypes (Diaz et al. 2016). For this reason, plant communities may be effectively described through their functional profile, allowing the comparison of ecosystems within a coherent framework. A successful scheme for the assessment of the functional strategies of plant species is Grime's Competitor–Stress tolerator– Ruderal (CSR) scheme (Grime 1977, reviewed by Grime and Pierce (2012). CSR theory predicts that the strategies of plant species are an adaptive response to a three-way trade-off in the investment of resources between the ability to compete with neighbors (competitive strategy, C), tolerate stress (stress-tolerant strategy, S), or survive disturbance (ruderal strategy, R). The morpho-functional traits of each species can be used to assess its life strategy in the form of coordinates on the C, S, and R axes (Pierce et al. 2017). This theory provides a functional interpretation of plant communities in different ecological conditions and has been successfully used in alpine grasslands and pastures (Caccianiga et al. 2006; Pierce et al. 2007; Li and Shipley 2017). The use of community-weighted mean (CWM) trait values (i.e. values weighted by species abundance at the plot level) allows the application of such an approach at the community level (Garnier et al. 2004; Dubuis et al. 2013) following the mass ratio hypothesis (Grime 1998), which suggests that the traits of the most abundant species have a proportionally higher role in ecosystem functioning. However, except for Moog et al. (2005) on semi-natural grasslands in Germany or Bahr et al. (2012) and Ejrnaes and Bruun (2000) on dry prairies in Denmark, the CSR approach has usually been used without considering soil characteristics; in general, studies have rarely considered the interactions of all the mentioned factors and their relative importance in shaping vegetation patterns.

The objective of this study was to provide a detailed assessment of ecosystem functioning based on quantitative and analytical measurements of soil chemical and physical parameters, biomass and productive measurements, floristic composition, and a 'functional summary' (Pierce et al. 2017) provided by an assessment of the CSR strategies; such an assessment was performed for an alpine pasture characterized by high soil and vegetation variability at a detailed scale to provide further insights into the capability of plant strategy variation to reflect the underlying ecological parameters and into the role of biodiversity in perspective of environmental changes.

2. Methods

2.1 Study site

The study site (Figure 1) is a 1.5 ha doline in Valchiavenna (Central Italian Alps, Lombardy; 46° 27′ 22″ N, 9° 21′ 05″ E), between 1920 and 1950 m in altitude, used as a cattle pasture under-loaded and grazed mainly by dairy cows, heifers, and calves.

The mean annual air temperature, measured by three meteorological stations located inside or very near the study area and characterized by different exposure types (north, plain, and south), is 3.1 °C; the mean annual precipitation is about 1300 mm (45%–50% as snow). During the vegetative period (June–September), the mean air temperature is 10.3 °C, the mean precipitation is about 600 mm, and the mean solar radiation is 19.3 MJ m^{-2} d^{-1}.

The doline shows strong topographic variability (maximum height difference of 30 m): in the northern part there is a rounded summit, the south-facing steep slope is subjected to water and wind erosion, while the flat bottom is sometimes subjected to water stagnation. Close to the doline at the west and east, respectively, there are a rolling plain surface covered by earth hummocks and a low-marked impluvium zone.

The study site has a carbonate (marble, crystalline limestone) substrate and a discontinuous felsic (mainly schist) glacial cover. Due to the different thicknesses of glacial deposits and because of slightly developed periglacial phenomena (earth hummocks, nivation, gelifluction, ploughing blocks) in the absence of permafrost, the soil characteristics are very variable and the pattern of vegetation is intricate.

The main soil types (IUSS Working Group WRB 2015) are Leptosols and Cambisols, to a smaller extent Umbrisols, and more rarely Podzols (Comolli et al. 2011).

The study area was characterized from the pedological, vegetation, and topographic points of view. Thirty-five sampling points were selected as representative of the main soil and vegetation types and of the main topographic features (Figure 1).

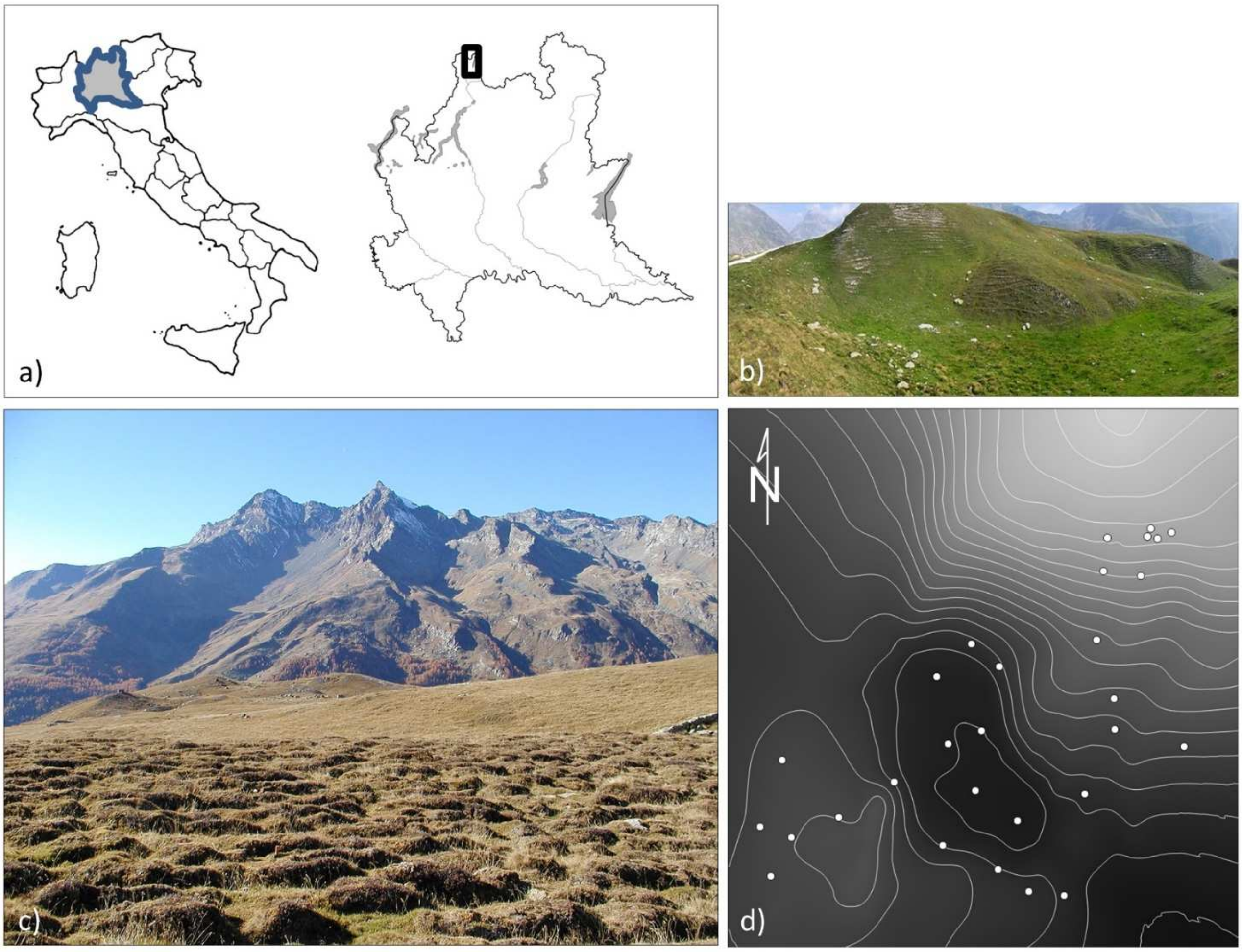

Figure 1: Study area: (a) geographic position of the study area; (b) the doline; (c) the earth hummock vegetation; (d) locations of the sampling points.

2.2 Vegetation sampling

A floristic survey was carried out at each sampling point to detect the occurrence and abundance of plant species and to outline the main plant communities. Vegetation sampling was performed during the growing season (20th July) through point-quadrat analysis. Thirty-five plots, each consisting of a 0.5 × 0.5 m quadrat with a 10 cm grid, were considered. At each node of the grid, the plant species touching the grid and the vertical line passing through the node were identified, and the number of contacts, which is a good indicator of relative abundance, was recorded. The overall number of contacts can also be considered a proxy for the biomass of the whole community (Redjadj et al. 2012). After the floristic survey, at each sampling point, a soil core sample was taken down to 10 cm (core diameter 10 cm; sampled volume 785 cm3) for determination of the root biomass. Extraction of roots from the cores was carried out by presoaking the sampled soil overnight in a solution of EDTA (40 g L−1) to facilitate washing. After that, roots were washed, picked out, separated into three diameter classes (< 2 mm; 2–5 mm; > 5 mm), and oven dried at 80°C for 24 h to determine the following dry masses of the root biomass, expressed as g dm−3: total (totRoots), < 2 mm diameter (very fine, vfRoots), 2–5 mm diameter (fine, fRoots). There were no roots with diameter > 5 mm. At 15 sampling point, roots were also sampled in the deeper layers (10–20 cm and 20–30 cm layers).

Twelve-year aboveground biomass data of the main types of the doline's vegetation are available; they were collected by mowing the grass at about 3 cm height within exclusion cages of 2 × 1 m. The mowing was performed at maximum vegetative development (between 15th and 30th July) and was repeated on the regrowth at the end of the season (September).

The functional profile of each vegetation type was evaluated by assessing for each species its Grime's CSR strategy through the approach proposed by Pierce et al. (2017). Such an approach allows the assessment of the competitive, stress-tolerant, and ruderal components of a species' strategy, expressed as a percentage, using a few leaf traits: the leaf area (LA), leaf dry matter content (LDMC), and specific leaf area (SLA). These parameters were calculated from the values of leaf fresh weight, leaf dry weight, and leaf area data obtained by previous studies (Caccianiga et al. 2006; Pierce et al. 2007; Pierce et al. 2017), an available comprehensive dataset (Cerabolini et al. 2010), and other data (Caccianiga and Cerabolini, unpublished).

For each sampling point, the CWM of the CSR average scores was calculated by weighting by the overall frequency of the observed species.

2.3 Soil sampling and laboratory analysis

Immediately after the vegetation sampling, besides the soil core sample for the determination of root biomass, another sample was collected down to 10 cm (core diameter 10 cm; sampled volume 785 cm3) for soil organic carbon (SOC) content and bulk density (BD) determination, and other soil analyses. Sometimes (11 cases for roots and 3 cases for SOC) the sample depth was shallower than 10 cm due to the presence of the fractured rock substrate near the soil surface.

On the collected soil samples, the following parameters were determined: pH in water (pHw; soil-to-solution ratio 1:2.5); SOC and total nitrogen (totN) by dry combustion with a Flash EA 1112 NC-Soil elemental analyzer (Thermo Fisher Scientific CN, Pittsburgh, USA) after removal of carbonates, if present, by hot HCl treatment; soil texture (four fractions) by sieving and sedimentation after dispersion with sodium hexametaphosphate; and available P (avP) according to Bray and Kurtz (1945). For BD determination, soil cores were oven dried at 105°C for 24 h and weighed; in the case of soils containing rock fragments (>2 mm diameter), the soil volume and mass were proportionally reduced to obtain the fine earth BD. The SOC content was also calculated on an area basis (Cstock, kgm−2, 0–10 cm depth) considering the soil BD and rock fragment volume.

The soil particle density (PD) was estimated according to PD (g cm−3) = 2.65 − 0.02SOM (%), where SOM (soil organic matter) was calculated as SOC (%) × 1.724. The total soil porosity (totPor) was calculated from PD and BD. Soil humus fractions were determined according to Anderson and Schoenau (2008) to obtain the carbon and nitrogen contents of fulvic acids (CFA, NFA), humic acids (CHA, NHA), and humin (CHUM, NHUM).

Throughout the vegetative season, the soil temperature at 5 cm depth and soil water content at 0–8 cm depth were measured every two weeks at each sampling plot, and the measurements were then averaged. The soil water content was measured using a portable TDR system (IMKO Micromodultechnik, GmbH, Ettlingen, Germany) and then converted (using the measured BD) to the water-filled pore space (WFPS).

The morphologic and topographic features of the doline were obtained from a digital terrain map (DTM) with a 2 m resolution.

The mean solar radiation for the vegetation period (June– September) was calculated for each sampling point in ArcGIS Desktop (ESRI 2011, Release 10) using the DTM.

After the topsoil sampling, a soil profile was opened at each of the 35 sampling points until a depth of about 100 cm or until the rock substrate. The soil was described and sampled by horizons (FAO 2006). Laboratory analyses were carried out on all the collected samples using the methods indicated above and, for Fe and Al fractionation only, using the official Italian methods (MiPAF 2000); soils were then classified using the WRB taxonomic system (IUSS Working Group WRB 2015). Based on the characteristics of the soil, taking into account the horizons A and B (thus excluding C and R), the available water content (AWC) was calculated for each sampled point; the empirical equations of Ghanbarian-Alavijeh and Millàn (2010) were used, considering the measured BD for the 0–10 cm layer and an estimated BD value for the underlying layer (Hallet et al. 1998). The intensity of water erosion was qualitatively evaluated on the basis of the bare soil percentage by direct observation and using remote sensing images.

The list of all investigated parameters and their abbreviations are shown in Table 1.

2.4 Statistical analysis

A clustering procedure was used to identify the main vegetation types using the Chord distance and Unweighted Pair-Group Method using arithmetic Averages (UPGMA) as a clustering method. In order to test the relationships between soil properties and vegetation types, the linear mixed model (LMM) procedure was performed (Bolker et al. 2009) to test for autocorrelation among the model residuals (Searle et al. 2009). If linear model assumptions on the residuals distribution were not satisfied, a Gaussian anamorphosis transformation (using ISATIS release 13.01 of software package; Geovariances 2013) of the response variable was performed. To compare the –2 log likelihood fitting criteria of different models (spatial, nonspatial), a likelihood ratio test was performed.

Statistical analyses were performed using PROC MIXED (Littell et al. 2006) of SAS (release 9.4, SAS Institute). The spatial covariance function of residuals was iteratively determined using the statement REPEATED by estimating the partial sill, range, and nugget effect parameters (Littell et al. 2006). The statistical difference between means was assessed through contrast analysis using the instruction LSMEANS.

The relationships between soil properties, environmental variables, and plant species were investigated using canonical correlation analyses (CCA; CANOCO version 4.5). Only the most abundant plant species (i.e. those with the highest number of recorded contacts) were included in the CCA. We selected the five most abundant species for each of the five vegetation types; as some species dominated in more than one vegetation type, a total of 14 species was included in the analysis. The Monte Carlo permutation test was performed in order to assess both the significance of the environmental variables and the ordination axes. To reduce the data set complexity and noise in the variance components, one from each pair of highly correlated variables was removed from the analysis (Perez-Riverol et al. 2017).

The CSR life strategy was also interpreted by principal component analysis (PCA; CANOCO version 4.5) mapping C, S, and R coordinates, soil and environmental variables, and vegetation types into the same space to investigate their correlations.

Table 1: Investigated soil properties, environmental variables, vegetation, and their abbreviations.

Soil properties, environmental variables and vegetation	Unit	Abbreviation
Soil organic carbon content of the 0-10 cm mineral soil layer	%	SOC
Soil organic carbon stock of the 0-10 cm	kg m^{-2}	SOC_{stock}
C:N ratio of the 0-10 cm mineral layer		CN
pH in water of the 0-10 cm mineral layer		pH_w
Available phosphorus content of the 0-10 cm mineral layer	mg kg^{-1}	avP
Bulk density of the 0-10 cm mineral layer	g cm^{-3}	BD
Coarse sand content of the 0-10 cm mineral layer	g kg^{-1}	cSand
Soil porosity	%	totPor
Silt content of the 0-10 cm mineral layer	g kg^{-1}	
Clay content of the 0-10 cm mineral layer	g kg^{-1}	
Water content	%	WFPS
Total, very fine and fine total root biomass	g dm^{-3}	totRoots, vfRoots, fRoots
carbon and nitrogen content of fulvic acids	g kg^{-1}	C_{FA}, N_{FA}
carbon and nitrogen content of humic acids	g kg^{-1}	C_{HA}, N_{HA}
carbon and nitrogen content of humin	g kg^{-1}	C_{HUM}, N_{HUM}
Slope	%	
Solar radiation	MJ m^{-2} d^{-1}	
Soil temperature at 5 cm depth	°C	T
Available water content	mm	AWC
Intensity of erosion		Eros
Reach pasture		RP
Earth hummocks		EH
Sesleria varia grassland		Sv
Bentgrass		B
Nardus stricta pasture		N

3. Results

3.1 Vegetation

The main vegetation types identified by cluster analysis were: 1. communities dominated by bentgrass (*Agrostis schraderiana*) (B); 2. *Nardus stricta* pastures (N); 3. rich pasture dominated by tall grasses and forbs (*Phleum pratense, Poa alpina, Alchemilla vulgaris, Deschampsia caespitosa*) (RP); 4. *Sesleria varia* grasslands (SV); and 5. earth hummocks (EH) (Table S1).

The number of contacts was the highest for RP, followed by EH, B, N and SV; the average number of species was higher for SV and N than for the other vegetation types (Figure 2 a and b).

The annual aboveground biomass production measured for the main vegetation types showed great differences (Figure 2c): RP vegetation produced more than the others; N pasture and earth hummocks gave intermediate biomass; while B and SV (considered together) were characterized by lower production. The average water content of the biomass was instead similar between the vegetation types (64% – 68%; data not shown). The totRoots biomass (about 80% represented by vfRoots) averaged to 4.9 g dm^{-3}, with a minimum of 1.8 and a maximum of 13.2 g dm^{-3}. Most of the roots, except for those from earth hummocks, which have a more homogeneous distribution with depth, were amassed in the first 10 cm (Table 2), with no statistical differences among vegetation types.

The distribution of Grime's life strategies showed small variations among the investigated communities (Figure 3), within which the species mainly exhibited a stress-tolerant attitude (Table S1): the bentgrass community was mainly composed of different stress tolerators, with *Agrostis schraderiana* dominant; in *Sesleria varia* grasslands, species mainly showed stress-tolerant (S) (*Sesleria varia, Carex caryophyllea, Poa alpina, Helianthemum oleandicum*) and stress-tolerant–ruderal (SR) (*Hippocrepis comosa*) strategies; earth hummocks were characterized by few species, mainly stress tolerators (*Nardus stricta, Agrostis schraderiana, Vaccinium uliginosum*) and SR (*Trifolium alpinum, Avenella flexuosa*); *Nardus stricta* pastures included species showing S strategy, with *Nardus stricta* dominant and *Festuca nigrescens* and *Agrostis schraderiana* much less frequent. Rich pastures were marked by a wider range of strategies, with dominance of the S species (*Agrostis schraderiana, Poa alpina, Festuca nigrescens, Deschampsia caespitosa*) and the presence of other species which mainly exhibited relatively generalist strategies, such as *Alchemilla vulgaris* and *Trifolium pratense*, with stress-tolerant– competitive (SC) and R attitudes, respectively.

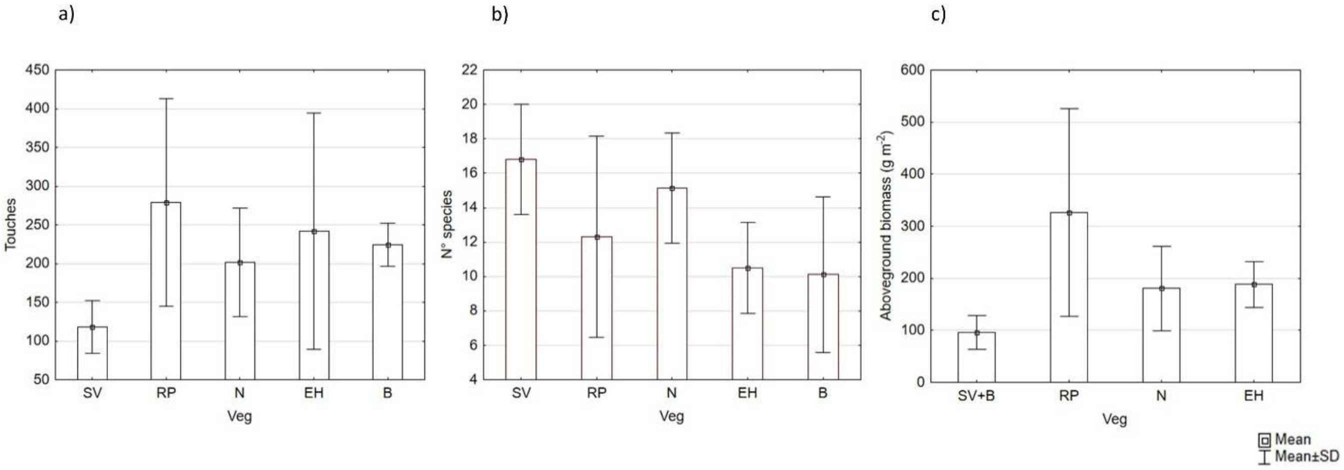

Figure 2: Vegetation types of the investigated doline: (a) number of contacts; (b) number of species; and (c) aboveground biomass (2006–2018). SV: *Sesleria varia* grassland; RP: rich pasture; N: *Nardus stricta* pasture; EH: earth hummocks; B: bentgrass.

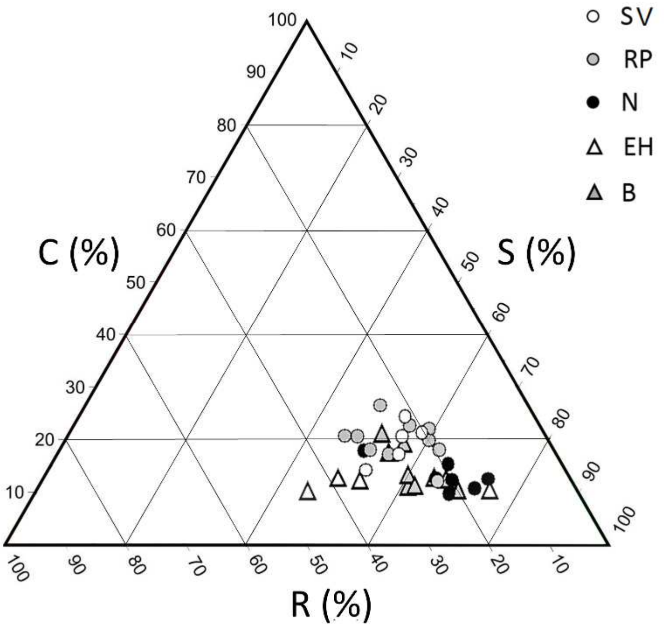

Figure 3: Functional profiling through Grime's CSR model (R: ruderal strategy; C: competitive strategy; S: stress-tolerant strategy) for each sampling point of the investigated vegetation types (SV: *Sesleria varia* grassland; RP: rich pasture; N: *Nardus stricta* pasture; EH: earth hummocks; B: bentgrass).

Table 2: Total root density (g dm^{-3}) for each vegetation type in the three investigated layers.

		0-10 cm			**10-20 cm**			**20-30 cm**	
Vegetation	N	Mean	Std. Err.	N	Mean	Std. Err.	N	Mean	Std. Err.
B - Bentgrass	9	5.17	0.80	3	1.56	1.26		-	-
RP - Rich pasture	10	5.35	0.70	4	0.99	0.60	4	0.93	0.30
S - *Sesleria varia* grassland	5	8.16	1.97	3	1.56	1.26		-	-
EH - Earth hummock	4	8.04	2.17	4	3.83	1.42	4	1.46	0.30
N - *Nardus stricta* pasture	7	6.20	0.95	3	0.99	0.28	3	1.39	0.28

3.2 Soil

The representative soil types which characterized the investigated doline are shown in Table 3. They were mainly Leptosols and Cambisols, all rich in SOC content in the topsoil. Leptosols, shallow and lowly developed soils, were mainly located along steeper slopes with southern exposure and limited by carbonate bedrock, which influenced their base saturation and reaction (Rendzic Leptosols; 10–15 cm thick; base saturation (BS) > 50%; pH > 6.0). Where slopes were still steep but marbles were slightly deeper, we found a few thin soils with thick (25–35 cm) surface horizon, low BS, and high SOC (Leptic Umbrisols).

The most widespread soil type at the study site was Cambisol, slight to moderately developed soil, which mainly differed in thickness and saturation status. In flat and moist areas of the lower part of the doline, these soils were thick (until 100 cm deep) and with high base saturation (Eutric Cambisols), while along the slight slopes and in the high plain part, not as wet as the bottom of the sinkhole, we mainly found thinner soils, desaturated in bases (Dystric Cambisols) and rich in rock fragments. In areas with thick glacial cover rich in highly weathered schist, Cambisols passed gradually towards Podzols, with evidence of cryoturbation caused in the past by frost action; these soils, classified as Dystric Cambisols (Protospodic) – Entic Podzols, were deep, very acid, with strong silt content and few rock fragments, more compacted, and with less organic matter than the other soils.

When considering only the first layer (0–10 cm depth, but slightly less for three points), the SOC and Ntot contents were high and very variable, ranging between 2.2% and 13.6% and 0.2% and 1.2%, respectively (Table 4); the average C/N ratio (± SE) was 10.9 ± 0.2. The average avP content was 23.6 ± 2.9 mg kg^{-1}; the surface soil texture was mainly sandy loam; and the pHw varied over a wide range, from very acid (4.3) to neutral (7.1) values.

The average soil temperature (at 5 cm depth) during the grazing season was 16.1 °C, ranging between 12.7 and 19.3 °C; the WFPS (mean ± SE) was 42.6% ± 1.58%, varying between 24.3% and 59.9%.

Table 3: Main properties of representative soil profiles (taxonomy according to IUSS Working Group WRB, 2015).

Horizon	Depth (cm)	Colour (moist)	Rock fragments	Structure	Roots	pH H_2O	SOC (g kg^{-1})	C:N ratio	Textural class	Notes
Soil profile #1 – Leptic Umbrisol (Siltic) (representative of bentgrass vegetation)										
A1	0-11	10YR 2/1	few, f	granular, f	common, vf-f	4.1	116	10.3	silty loam	
A2	11-27	10YR 3/3	many, m-f	sub. blocky, f-m	very few, vf-f	5.0	54	8.9	silty loam	
R	27+									fractured marble
Soil profile #2 – Dystric Leptic Cambisol (Humic) (representative of *Nardus stricta* pasture)										
A	0-7	10YR 2/1	absent	granular, f	common, vf-f	4.9	126	11.6	loamy sand	
BAw1	7-15	10YR 3/2.5	common, vf-f	sub. blocky, f	few, vf-m	4.7	40	10.4	silty loam	
BAw2	15-42	10YR 3.5/4	common, vf-f	sub. blocky, f	few, f	5.3	27	11.5	sandy loam	
R	42-60		abundant, vf-f	single grain	very few, vf					fractured marble
Soil profile #3 – Eutric Cambisol (Humic, Loamic) (representative of rich pasture vegetation)										
Ah	0-5	10YR 2/1	absent	granular, f	common, vf-f	5.7	82	10.5	loam	earthworms
A	5-15	10YR 3.5/3	few, vf-f	granular, m	very few, mf-f	5.5	31	9.2	loam	
Bw1	15-26	10YR 4/4	few, vf-f	sub. blocky, f-m	very few, vf	5.7	15	10.0	loam	
Bw2	26-34	2.5Y 4.5/4	few, vf-f	sub. blocky, f-m	very few, vf	6.0	14	9.8	loam	
C1	34-55	2.5Y 5/4	common, vf-m	massive	very few, vf	5.9	3		loam	
C2	55-95	2.5Y 5/3	common, vf-m	massive	absent	6.0	2		loam	
Soil profile #4 – Eutric Lithic Leptosol (Humic) (representative of *Sesleria varia* grassland)										
A1	0-3	10YR 2/1	common, vf-f	granular, f	many, vf	6.3	114	9.8	sandy loam	
A2	3-8	10YR 2/1	frequent, vf-m	granular, f	few, vf	6.7	70	9.3	sandy loam	total carbonates: 3 g kg^{-1}
R/A	8-40	10YR 2/3	abundant, f-vc		very few, vf	7.2	62	9.1		fractured marble; total carbonates: 42 g kg^{-1}
Soil profile #5 – Dystric Cambisol (Humic, Siltic, Relictiturbic) (representative of earth hummocks vegetation)										
A	0-5	10YR 3/3	absent	granular, m	common, vf-m	4.5	43	10.2	silty loam	
AB	5-11	10YR 3.5/4	absent	granular, m	few, vf-m	4.3	29	10.2	silty loam	
Bw	11-30	10YR 5/5	few, vf	sub. blocky, m	few, vf-m	4.4	11	8.5	silty loam	
CB1	30-55	10YR 5/6	few, vf	platy (lithogenic)	very few, vf-f	4.7	1		silty loam	
CB2	55-80	1Y 5/6	common, vf	platy (lithogenic)	very few, vf	5.9	0		silty loam	
C	80-120	2.5Y 5.5/6	many, vf-m	massive	absent	7.6	0		sandy loam	total carbonates: 351 g kg^{-1}

Legend for rock fragments, structure and roots: vf: very fine; f: fine; m: medium; c: coarse; vc: very coarse. Textural classes according to Soil Survey Division Staff (1993).

Table 4: Main statistics of soil properties in the 0–10 cm layer. For abbreviations and units of variables, see Table 1.

	N	Mean	Median	Minimum	Maximum	Std. Dev.	Std. Error	Coeff. Var.(%)
Slope	35	22.31	25.00	0.00	51.00	18.11	3.06	81
BD	35	0.71	0.70	0.41	1.02	0.14	0.02	20
totPor	35	70.06	70.66	60.29	81.14	5.09	0.86	7
pHw	35	5.1	4.8	4.3	7.1	0.7	0.1	14
SOC	35	8.0	7.8	2.2	13.6	2.7	0.4	34
totN	35	0.8	0.8	0.2	1.2	0.3	0.1	36
CN	35	10.9	10.9	8.7	14.2	1.1	0.2	10
CFA	35	11.56	11.45	4.85	17.51	2.86	0.48	25
CHA	35	20.95	21.18	5.11	32.36	6.91	1.17	33
CHUM	35	41.19	40.34	12.51	80.76	16.06	2.71	39
NFA	35	1.14	1.07	0.48	1.69	0.32	0.05	28
NHA	35	2.00	1.99	0.27	3.25	0.72	0.12	36
NHUM	35	3.35	3.14	0.76	6.77	1.52	0.26	45
avP	35	23.57	20.41	2.86	67.48	17.36	2.93	74
Sand	35	556	582	355	781	89.09	15.06	16
Silt	35	372	363	178	554	81.42	13.76	22
Clay	35	72	72	41	106	15.41	2.61	21
T	35	16.1	15.9	12.7	19.3	1.9	0.32	12
WFPS	34	42.6	42.5	24.3	59.9	9.2	1.58	22

3.3 Soil–plant interactions

At the top of the slope, where soil was poorly developed, thin, and limited by highly fractured bedrock, the typical vegetation was *Sesleria varia* grassland (Figure 4). The southfacing slope was moderate to high in terms of gradient (25%–51%) and soil erosion was often strong (activated by cattle grazing, but mainly due to runoff and snow and wind erosion); here, the distribution pattern of *Sesleria varia* and *Agrostis schraderiana* communities was very intricate, with patches often less than 1-meter-wide, making their separation difficult. However, soils under *Sesleria varia* grassland (Rendzic Leptosols) usually differed from those under bentgrass (Leptic Umbrisols) showing a neutral instead of acidic reaction and higher total porosity due to their low bulk density. In flat or gently sloped areas, particularly at the concave bottom of the doline, the prevailing vegetation was rich pasture, and soils were mainly deep Eutric Cambisols with intense biological activity by earthworms in the topsoil. Along water flow zones and near flat areas, *Nardus stricta* pastures spread over moderately deep and sometimes thin Dystric Cambisols, with low pH and common rock fragment content. In areas with weathered shale substrate, where soils were very acidic and deep, there was a significant presence of earth hummocks, with a pattern of small reliefs and depressions (about 40 cm difference between the top and bottom of the hummocks); the depressed areas were strongly compacted by cattle transit, resulting in water stagnation following prolonged rainy events.

Concerning surface soil characteristics, SV and EH greatly differed from the other vegetation types (Figure 5 and Table S2). The *Sesleria varia* community significantly ($p < 0.05$) differed in terms of pH (the highest values), BD, and clay content (the lowest values) from the other vegetation groups; it exhibited, in common with EH, lower avP than the other vegetation types. EH was instead characterized by significantly ($p < 0.05$) higher BD, CN, and silt and lower SOCstock, CHA, and CFA than the other communities. The vegetation types N, RP, and B mainly had similar values for most of the investigated parameters, with intermediate values for CN, BD, pH, and textural fractions and highers value of SOCstock, CHA, and CFA than EH and SV. Concerning the soil water content, the SV and B vegetation types were significantly drier than N and RP, while EH showed intermediate WFPS values.

The parameter with the highest discriminating power was SOCstock. B, RP, and EH significantly differed from each other, with the highest values for B and the lowest values for EH; RP showed intermediate values, similar to SV and N.

The CCA resulted in high eigenvalues and cumulative percentage variances of the species–environment data, which were indicative of distinctive species assemblages across the different vegetation types (Table 5 and Figure 6); the cumulative constrained variability explained by the first two axes was 60.7%. The species–environmental parameter correlations were 0.965 for axis 1 and 0.880 for axis 2. The main identified ecological gradient was that of soil pH, temperature, and erosion risk, marked by base-requiring species such as *Sesleria varia, Helianthemum oleandicum, Hippocrepis comosa*, and *Carex caryophyllea*. Axis 2 identified a second gradient coinciding with higher C/N ratio, BD, and silt content and lower Cstock with dominant *Vaccinium myrtillus* and *Avenella flexuosa*; to a lesser extent, *Trifolium alpinum; Phleum alpinum*, and *P. pratense* seem to be linked to soils characterized by higher water content and avP.

We conducted PCA to compare the CSR strategies with respect to soil and environmental properties. The first two factors explained 61% of the total variance: Factor 1 accounted for 41% and Factor 2 for 20%. A distinction between conditions favoring ruderal, competitive, and stress-tolerant components could be observed (Figure 7). The ruderal component was associated to conditions favoring slow organic matter mineralization (high C/N) such as those in the earth hummock areas, while competitiveness was positively correlated with pHw. The stress-tolerant component was weakly correlated with WFPS and avP characterizing the resting areas. A clear main gradient of soil variables with respect to CSR strategies could not be observed; the investigated plant communities share an overall common stress-tolerant profile linked to regional environmental conditions, while, at small scale, specific local factors act as different stress sources (see discussion).

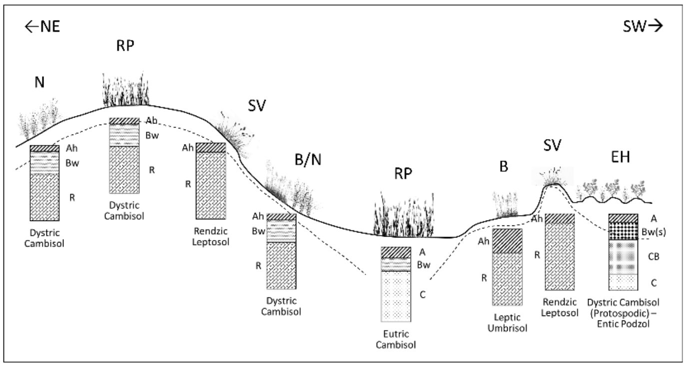

Figure 4: Schematic representation of soil–plant relationships along a topographic transect in the investigated doline. The dotted line approximately represents the depth of the substrate. B: bentgrass; RP: rich pasture; SV: *Sesleria varia* grassland; EH: earth hummocks; N: *Nardus stricta* pasture.

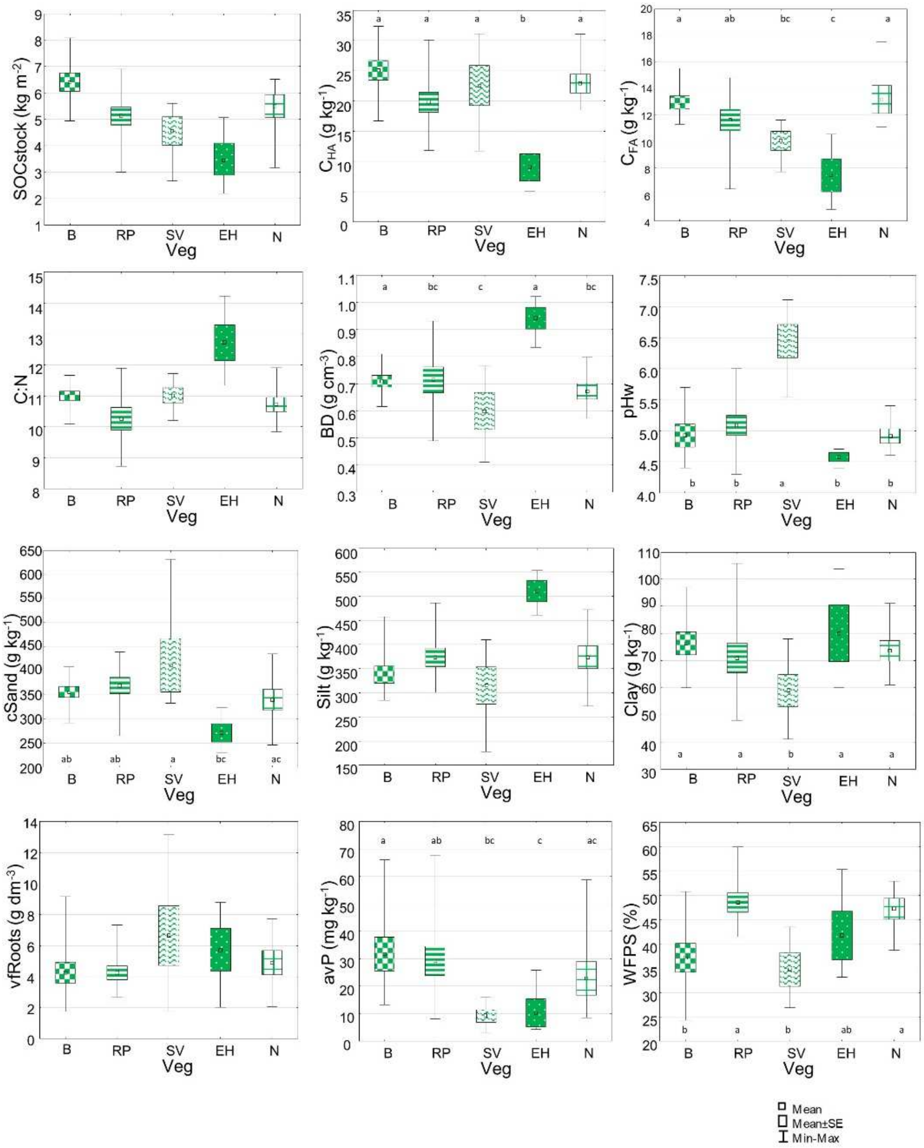

Figure 5: Box plots for the comparison of soil properties among vegetation types (B: bentgrass; RP: rich pasture; SV: *Sesleria varia* grassland; EH: earth hummocks; N: *Nardus stricta* pasture). Different letters indicate statistically significant differences ($p < 0.05$) in the response variable among vegetation types in the mixed model. For abbreviations of variables, see Table 1.

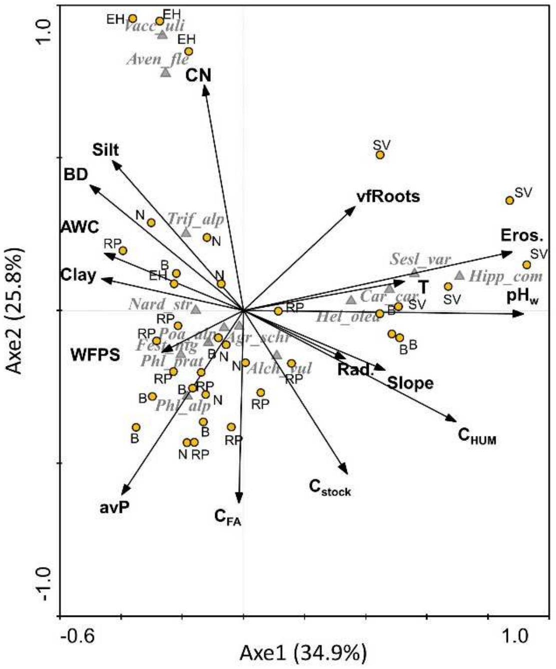

Figure 6: CCA analysis of plant species in relation to the considered soil and environmental variables. Significant variables (at $p < 0.05$) are reported within rectangles. Species abbreviations: *Agrostis schraderiana*: Agr_schr; *Alchemilla vulgaris*: Alch_vul; *Carex caryophyllaea*: Car_car; *Festuca nigrescens*: Fest_nig; *Heliantemum oleandicum*: Hel_olea; *Hippocrepis comosa*: Hipp_com; *Nardus stricta*: Nard_str; *Phleum alpinum*: Phl_alp; *Phleum pratense*: Phl_prat; *Poa alpina*: Poa_alp; *Sesleria varia*: Sesl_var; *Trifolium alpinum*: Trif_alp; *Vaccinum uliginosum*: Vacc_uli. For abbreviations of variables, see Table 1.

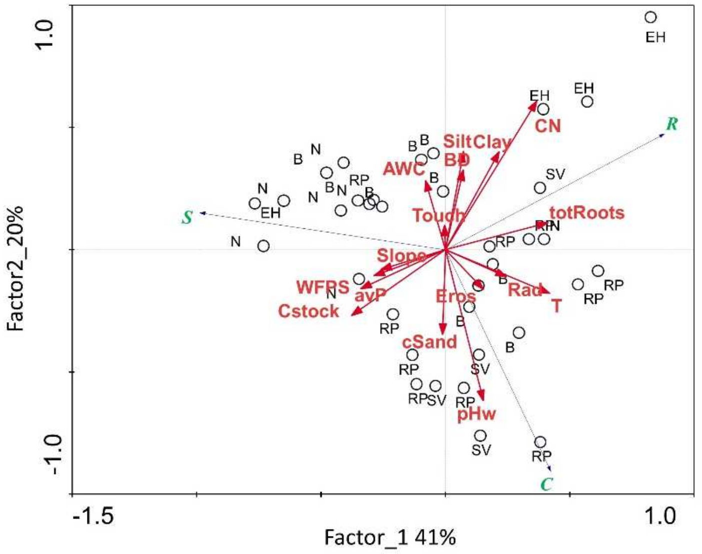

Figure 7: PCA analysis. Plot of CSR components, considered soil and environmental variables, and vegetation types. For abbreviations of variables, see Table 1.

Table 5: Canonical correlation analysis (CCA) for the 14 most abundant plant species in relation to the considered environmental variables (soil properties of the 0–10 cm layer).

	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalues	0.484	0.358	0.193	0.111
Species-environment correlation	0.965	0.880	0.778	0.808
Cumulative percentage variance of species data and species-environment relation	34.9	60.7	74.6	82.7
Variable	**Lambda 1**	**Lambda A**	**P**	**F**
pHw	**0.42**	**0.42**	***	**8.62**
CN	**0.23**	**0.24**	***	**5.74**
Eros	**0.41**	**0.14**	***	**3.79**
Silt	**0.21**	**0.11**	**	**2.95**
WFPS	**0.12**	**0.07**	*	**2.00**
Rad	**0.11**	**0.07**	**	**2.07**
T	**0.17**	**0.06**	*	**1.88**
Cstock	0.18	0.06	ns	1.66
HUMC	0.3	0.04	ns	1.33
avP	0.23	0.04	ns	1.27
Slope	0.19	0.04	ns	1.14
Clay	0.13	0.03	ns	1.13
FAC	0.17	0.03	ns	0.89
AWC	0.12	0.02	ns	0.65
BD	0.2	0.02	ns	0.44

4. Discussion

We selected an alpine pasture that exhibited a wide range of plant communities and soil conditions. This study showed evidence of strong relationships between vegetation (floristic composition, vegetation types, and life strategies) and soil chemical (pHw, avP, SOC, CN) and physical (BD, AWC, texture) properties, climatic and pedoclimatic parameters (T, WFPS, Rad, AWC), topography (slope, erosion), and livestock grazing.

It has long since been demonstrated that mutual soil–plant relationships produce spatial patterning in soil properties and that individual plant performance and plant communities may respond to soil variability (Rubio and Escudero 2000; Casa and Castrignanò 2008; Ferré et al. 2014). In mountain areas, such variability is exacerbated by heterogeneity in the geomorphology and lithology of the soil parent material. In accordance with this, changes along the topographic gradient from dry calcareous to damp acidic soils, reflecting variations in soil fertility and conditions of stress and disturbance, were observed.

The investigated doline mainly exhibited stress-tolerant strategies, although the kinds of stress were different. The overall, background S strategy of the investigated communities is probably linked to the climatic features of the investigated area, a temperature-limited high-altitude site with short growing season. At local scale, soil, topographic and grazing variables act as specific stress and/or disturbance factors, providing the specific features of each plant community (Pierce et al. 2007). In the upper part of the south-facing slope, on shallow soils, stress related to sub-alkaline reactions and dry conditions favored the presence of *Sesleria varia, Carex caryophyllea, Poa alpina*, and *Helianthemum oleandicum*. Biomass production was restricted, but biodiversity was high, in accordance with the ‘unimodal diversity–productivity relationship’ or ‘humped-back model’ which implies that the highest levels of biodiversity occur at intermediate levels of productivity rather than at the highest ones (Grime 2006; Adler et al. 2011). Where disturbances caused by erosion and cattle transit were higher, species with the stress-tolerant–ruderal (SR) strategy appeared. Grazing is known to have the potential to modify ecosystems and change their structure and function (Hobbs et al. 1996) – in this case, by promoting erosion through mechanical disturbance of the soil surface (Pietola et al. 2005).

With decreasing slope, in particular along the water flow areas, another stress-tolerant species, *Nardus stricta,* prevailed; this species is often dominant under acidic and nutrient- poor conditions (Landolt et al. 2010) and, thus, in communities characterized by low productivity, forage quality, and palatability. Bentgrass communities occurred nearby on convex slopes and, thus, under intermediate topographic conditions between *Nardus stricta* communities and those linked to steeper slopes. These communities share the overall ecological profile of Nardus-dominated pastures, with a slight displacement towards the C and R corners of the CSR triangle. For both communities, the dominant S strategy is an expected consequence of low productivity, the main driver of stress tolerance syndrome (Grime 2006).

In the flat zones at the bottom of the doline, where soils were deep, rich in nutrients, and damp, species with stresstolerant (S), stress-tolerant–competitive (SC), and stresstolerant– ruderal (SR) attitudes grew. Rich pastures were characterized by high biomass and a low number of species with high nutrient acquisition, high photosynthetic efficiency, and fast growth (Grime 2001); such species usually form tall and uniform stands with plastic biomass allocation to leaves and roots to maximize nutrient acquisition. Here, livestock rest, which increases the nutrient turnover rates and selective grazing, trampling, and soil compaction (Manier and Hobbs 2007; Jones et al. 2010). Patchy concentrations of nutrients, together with local stress factors such as temporary water stagnation, provide contrasting microsites for a range of life strategies; thus, dominant species include both S strategists, such as *Festuca nigrescens*, and species with a relatively high C component, like *Phleum pratense*. However, it should be considered that in spite of being the most productive and exhibiting the highest C component, these communities share the overall S strategy of the whole ensemble of investigated plant communities.

Despite the high aboveground productivity, the root biomass in the investigated surface layers of the rich pastures was not different from that of the other vegetation types. Cambisols of the stable areas were deep, but most of the roots were in the surface layer, and only a small part was deeper (10–30 cm); this is in accordance with what was found for tundra (an environment comparable with ours), which was characterized by 80%–90% of the root mass occurring in the upper 0.3 m of the profile (Jackson et al. 1996). The lower root/shoot ratio in this community is probably linked to its higher nutrient status with respect to the other investigated communities, as this ratio is expected to increase under low nutrient availability because of greater allocation of nutrients to root growth and increased root longevity (Chapin 1980).

The earth hummocks represented a separate case; this vegetation type developed above acidic soils in the level or nearlevel areas with low drainage and characterized by an alternation of raised and depressed areas due both to periglacial phenomena and livestock trampling, to which corresponded an alternation of stress-tolerant–ruderal and stress-tolerant strategies. The raised areas were characterized by shrub species of the subalpine heathlands (*Vaccinium uliginosum*), *Trifolium alpinum*, and high cover of *Avenella flexuosa,* to which the ruderal component is mainly due. The depressed areas, more compacted due to livestock transit and, thus, often damp after rainy events, showed flora which was instead comparable to that of a typical *Nardus stricta* grassland, with species tolerant to trampling and to high water availability, such as *Agrostis schraderiana*.

5. Conclusions

Overall, this study demonstrated the high spatial heterogeneity of soil properties and vegetation related to high variability in the topography, soil parent material, and pedoclimate. Moreover, vegetation pattern proved to be a valid indicator of environmental spatial variability resulting from all the factors working together; considering its spatial heterogeneity, and making use of its explanatory power for soil and morphology changes, it could be used to evaluate most of the biogeochemical processes related to the soil–vegetation complex. The use of CWM values effectively summarizes the overall ecosystem properties, even if they do not necessarily reflect the unique possible optimal strategies of the respective communities (Muscarella and Uriarte 2016), and a wide spectrum of strategies may occur in each plot, enhanced by the high small-scale variability of environmental parameters.

Our results highlight the importance of the use of complementary approaches to vegetation study, from quantitative data of the above- and belowground biomass to qualitative information provided by detailed floristic survey and functional approaches that may help to shed light on the processes underpinning community arrangement and functioning.

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7. Supplementary material

Table S1: Vegetation types, CSR values (%) and CSR strategy class for each investigated point.

Point	Veg. Type	C	S	R	CSR class
92	S	25.4	54.0	20.6	S
55	S	22.1	58.4	19.5	S
67	S	21.6	55.2	23.3	S
33	S	18.3	56.1	25.6	S
68	S	15.0	52.2	32.7	S
16	RP	27.0	48.9	24.1	CS
106	RP	22.8	56.0	21.2	S
29	RP	21.6	60.0	18.4	S
RP2	RP	20.4	47.8	31.8	SR
RP1	RP	20.2	46.3	33.5	SR
32	RP	20.1	60.7	19.1	S
39	RP	17.8	62.9	19.3	S
RP3	RP	17.2	52.5	30.3	S
105	RP	16.7	55.7	27.6	S
RN3	RP	12.0	66.0	22.0	S
44	N	17.5	51.2	31.2	S
86	N	15.4	66.2	18.4	S
70	N	12.1	66.3	21.6	S
RN1	N	11.8	68.6	19.6	S
53	N	11.8	74.6	13.6	S
90	N	10.1	73.5	16.4	S
RN2	N	9.6	69.0	21.4	S
49	EH	12.0	49.4	38.6	SR
37	EH	11.4	53.0	35.6	S
111	EH	9.6	75.9	14.5	S
36	EH	9.4	45.2	45.4	SR
RS1	B	21.4	52.1	26.5	S
RS2	B	19.1	56.6	24.3	S
RS3	B	17.6	55.2	27.2	S
96	B	13.1	60.3	26.6	S
102	B	12.5	65.2	22.3	S
103	B	11.7	67.3	21.0	S
13	B	11.1	62.5	26.3	S
40	B	11.1	61.6	27.3	S
8	B	9.7	70.3	20.0	S

R: Rich pasture; EH: Earth hummocks; SV: *Sesleria varia* grassland; B: Bentgrass; N: *Nardus stricta* pasture.
S: stress-tolerant strategy ; C: competitive strategy; R: ruderal strategy; SC: stress-tolerant-competitive strategy ; SR: stess-tolerant-ruderal strategy.

Table S2. Mean estimates and their standard error of the fixed effects. The response variables are the soil and environmental variables; the fixed effect is vegetation type. For abbreviations of variables, see Table 1.

Sp. (Gau): residuals were spatial correlated and the spatial covariance function of residuals was the Gaussian model; Non-sp.: residuals were non-spatial correlated; g defines gaussian variable

* Different letters denote statistically significant (p value < 0.05) differences between vegetation types.

\+ Different letters denote statistically significant (p value < 0.1) differences between vegetation types.l

Variables	Model Type	Statistics	B	EH	N	RP	S
SOCstock	Non-sp	estimate	6.403	3.450	5.499	5.123	4.564
		SE	0.379	0.568	0.429	0.359	0.508
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	a	c	ab	b	bc
gC_{HUM}	Sp. (Gau)	estimate	-0.100	-1.403	0.201	0.121	-0.130
		SE	0.338	0.558	0.342	0.363	0.411
		Pr > |t|	a	a	a	a	a
C_{FA}	Non-sp.	estimate	12.940	7.437	13.165	11.602	10.028
		SE	0.778	1.167	0.882	0.738	1.044
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	a	c	a	ab	bc
C_{HA}	Non-sp.	estimate	25.10	9.0	22.90	19.81	22.55
		SE	1.76	2.65	2.00	1.67	2.37
		Pr > |t|	<.0001	<.0001	<.0001	.0002	<.0001
		*	a	b	a	a	a
totN	Non-sp.	estimate	0.832	0.300	0.814	0.737	0.864
		SE	0.074	0.111	0.084	0.070	0.099
		Pr > |t|	<.0001	.0116	<.0001	<.0001	<.0001
		*	a	b	a	a	a
gCN	Non-sp.	estimate	0.075	1.415	-0.182	-0.559	0.106
		SE	0.279	0.418	0.316	-0.559	0.106
		Pr > |t|	.7888	.0020	.5679	.0433	.7792
		*	b	a	b	b	b
pHw	Non-sp.	estimate	4.92	4.57	4.91	5.09	6.44
		SE	0.16	0.24	0.18	0.15	0.21
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	b	b	b	b	a
cSand	Sp. (Gau)	estimate	370	279	319	381	397
		SE	27.66	40.67	25.04	25.47	33.90
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	ab	bc	ac	ab	a
Silt	Non-sp.	estimate	338	511	374	373	315
		SE	21.2	31.8	24.0	20.1	28.4
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	b	a	b	b	b
Clay	Non-sp.	estimate	76	80	73	71	59
		SE	5.0	7.5	5.6	4.7	6.7
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	a	a	a	a	b
gBD	Non-sp.	estimate	0.014	1.485	-0.265	-0.038	-0.766
		SE	0.274	0.411	0.310	0.259	0.376
		Pr > |t|	.9583	.0011	.3992	.8847	.0458
		*	b	a	bc	bc	c
avP	Sp. (Sph)	estimate	34.2	9.65	25.9	31.5	18.4
		SE	6.0	9.0	5.2	5.3	6.8
		Pr > |t|	<.0001	.3005	<.0001	<.0001	.0116
		*	a	c	abc	ab	bc
vfRoots	Non-sp.	estimate	4.27	5.73	4.91	4.25	6.65
		SE	0.81	1.22	0.92	0.77	1.09
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		+	b	ab	ab	b	a
		estimate	-0.077	0.310	-005	-0.159	0.218
gfRoots	Non-sp.	SE	0.342	0.513	0.387	0.324	0.459
		Pr > |t|	.8217	.5503	.9883	.6278	.6383
		+	b	a	ab	ab	ab
		estimate	0.633	-0.452	-0.659	-0.150	0.444
gRad	Non-sp.	SE	0.297	0.446	0.337	0.282	0.398
		Pr > |t|	.0414	.3183	.0601	.5987	.2744
		*	a	ab	b	ab	a
		estimate	0.157	-0.402	-0.121	-0.351	0.912
gT	Non-sp.	SE	0.311	0.467	0.353	0.295	0.417
		Pr > |t|	.6185	.3956	.7333	.2440	.0370
			a	a	a	a	a
		estimate	37.1	41.7	47.2	48.5	34.7
WFPS	Non-sp	SE	2.5	3.8	2.9	2.4	3.4
		Pr > |t|	<.0001	<.0001	<.0001	<.0001	<.0001
		*	b	ab	a	a	b
		estimate	23	1	39	11	37
slope	Non-sp.	SE	4.2	6.3	4.8	4.0	5.6
		Pr > |t|	<.0001	.9071	<.0001	.0111	<.0001
		*	b	c	a	c	ab

Community-level variation in plant functional traits and ecological strategies shapes habitat structure along succession gradients in alpine environment

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Community Ecology 21: 55–65
https ://doi.org/10.1007/s4297 4-020-00012 -9

Abstract

Plant traits and ecological strategies elucidate various aspects of ecosystem functioning and services. However, the well-recognized trade-offs evident at the species level are not always expected to mirror community-level variation. Here, we investigated, at the regional scale, the community-level trade-offs of three key plant traits representing economics and size spectra (LA - leaf area, LDMC - leaf dry matter content and SLA - specific leaf area) and Grime's CSR (competitive, stress tolerant, ruderal) plant strategies. We compared six siliceous alpine plant communities, also representative of Habitat types of EU Community interest (Habitats Directive, 92/43/EEC), distributed along a succession gradient, using a wide range of phytosociological relevés for which we calculated community weighted mean (CWM) trait values and C-, S- and R- scores. Our aims were to: (1) determine the validity of the plant community global spectrum of trait variation within alpine habitats; (2) investigate the discriminating capacity of plant traits and strategies to identify functional niches of dominance, stress and disturbance along the succession gradient; (3) quantify the variation in community structure (species richness and total species cover) through such functional niches. We observed a clear pattern of community-level trait variation that reflected the plant economics spectrum: from acquisitive and fast-growing characteristics in pioneer succession stages, to conservative and stress-tolerant features toward the succession climax, while the productive niche typical of *C*-selected strategies was scarce. Species richness and total species cover were both greater at intermediate levels of *S*- and *R*-selection gradients, indicating high niche differentiation in habitats characterized by exposure to stress or disturbance. Overall, this study demonstrates that trait trade-offs between communities identified at the global scale can undergo adaptation at the regional scale caused by local environmental conditions and also confirms the applicability of CSR strategies to investigate community-level variation of alpine vegetation.

Keywords: Community weighted mean, CSR theory, Functional identity, Functional niche, Habitats directive, Natura 2000

1. Introduction

Understanding the forces driving plant community functions has been one of the major goals of vegetation ecology over recent decades. These mechanisms have typically been investigated by analyzing plant functional traits, which are known to provide deeper insights into ecosystem functioning than approaches based solely on species identity (Díaz and Cabido 2001; McGill et al. 2006; Dubuis et al. 2013). Plant functional traits are defined as any morphological, physical or phenological features measurable at the individual level that affect the fitness of the individual (Violle et al. 2007). The main plant trait spectra that have been globally identified represent the trade-offs between resource economics and the size of plants and their organs (Díaz et al. 2016). Recently, by adopting the mass-ratio hypothesis (i.e., weighting trait values by species abundance; Grime 1998), it was confirmed that half of the global trait variation at the community level reflects such trade-offs (Bruelheide et al. 2018). Bruelheide et al. (2018) even stressed that such community trade-offs are weakly associated with climate and soil conditions at the global scale, and that trait combinations are predominantly filtered by local-scale factors. Hence, evaluating the consistency of these global-scale observations in well-defined environments at the regional scale could provide useful elements for the interpretation of trait variation within plant communities.

Many plant traits have a restricted geographic and phylogenetic coverage (Díaz et al. 2016), whereas three leaf traits are applicable to all vascular plant life forms worldwide and represent a wide range of plant functioning: leaf area (LA), determinant of the capacity to intercept light; leaf dry matter content (LDMC), associated with nutrient retention within the plant; specific leaf area (SLA), related to assimilation and growth rates (Garnier et al. 2017). LA is an indicator of the size spectrum, while LDMC and SLA describe opposite extremes of the plant economics spectrum (Díaz et al. 2016). For these reasons, LA, LDMC and SLA are also used to calculate plant CSR (competitive, stress tolerant and ruderal) strategies (Grime 2001), by integrating both plant economics (typical of S to R strategy selection) and plant size (typical of the C strategy) variation spectra in a three-way trade-off in plant functioning (Pierce et al. 2017).

Crucially, C, S and R values are not calculated directly from LA, SLA and LDMC: it is the trade-off between these traits, compared against the global spectrum (position along multivariate axes based on the global ranges of trait values), from which the CSR value is calculated, providing an absolute quantitative comparison with the global flora (Pierce et al. 2017). CSR analysis has allowed strategies to be determined for a wide range of vascular plant species from habitats worldwide, and many examples have been produced using the concepts of interspecific variation and of the 'strategy of the single species' (e.g., Cerabolini et al. 2010; Negreiros et al. 2014; Good et al. 2019; Rosenfield et al. 2019). In contrast, an approach involving the 'community strategy' or functional signature of the vegetation as a whole is much less evident (Hunt et al. 2004; Ciccarelli 2015; Ricotta et al. 2015; 2016; Cerabolini et al. 2016; Li and Shipley 2017), but could provide key insights into the variability of adaptations within plant communities.

This goal could be achieved, for example, by investigating the functional signature of plants along gradients of vegetation succession, which is an approach often used to gain a better understanding of the variation of plant traits along environmental gradients (Weiher et al. 2011), or to use these to predict community composition (Shipley et al. 2006). The CSR scheme has already been tested in alpine habitats along vegetation primary succession, as well as following zooanthropogenic modifications of climax (i.e., anthropogenic disturbances involving domesticated animals, such as cattle grazing), although only at the species level or at a local scale (Caccianiga et al. 2006; Pierce et al. 2007b; Pierce et al. 2017; Ricotta et al. 2015; 2016). However, the consistency of the CSR scheme at the community level over a regional scale has not yet been tested, despite promising recent results such as those arising from manipulation experiments of herbaceous mesocosms along a gradient of stress and disturbance (Li and Shipley 2017). Thus, it remains crucial to understand patterns of community variation of plant traits and strategies along environmental gradients such as those underpinning vegetation successions.

Mean values of traits and strategies along environmental gradients can be representative of functional niches (Violle et al. 2009; Pierce and Cerabolini 2018), which in turn could be used to investigate community structure, in terms of the mechanisms determining species richness and total species cover. Indeed, even though several phenomena control these two properties in herbaceous vegetation, they can be generalized in the form of the humped-back model of specie richness and biomass production (Grime and Pierce 2012). Productive environments favor species that dominate via competitive pre-emption of resources, leading to a reduction of species richness, while stress and disturbance initially stimulate diversity by suppressing potential competitors, but beyond a certain point, only highly specialized organisms can survive, again reducing species richness. Thus, at intermediate productivities, there is a greater range of potential opportunities (Pierce 2014) at which greater variation in functional trait values, CSR strategies and species richness have been demonstrated (Fraser et al. 2015; Cerabolini et al. 2016).

In the present study at the regional scale, we investigated the extent of community-level variation of key plant traits (LA, LDMC and SLA) and Grime's CRS ecological strategies of six alpine plant communities along a succession gradient. Communities were also representative of three Habitat types of EU Community interest (Habitats Directive, 92/43/EEC): 8110 'Siliceous scree of the montane to snow levels (*Androsacetalia alpinae* and *Galeopsetalia ladani*),' which represents early successional stages; 6150 ‘Siliceous alpine and boreal grasslands’ and 4060 ‘Alpine and Boreal heaths’, respectively, including middle and late successional stages. Specifically, we aimed to:

1) Determine whether the functional characterization of alpine plant communities agrees with the major axes of variation within the global spectrum of vegetation communities (Bruelheide et al. 2018);
2) Examine the discriminating capacity of plant traits and strategies at the community level to identify well-defined functional niches involving competition, stress and disturbance along the succession gradients;
3) Quantify the changes in communities structure, in terms of species richness (as a proxy of taxonomic diversity) and total species cover (sum of species cover as a proxy of biomass, Ónodi et al. 2017) within the functional niches identifiable along the succession gradient.

2. Methods

2.1 Study area

The vegetation relevés considered in this study were all located in the central-eastern Italian Alps, in the administrative region of Lombardy (Northern Italy), and spanned a surface area of approximately 3300 Km2 between latitude 45°35′–46°33′ N and longitude 9°17′–10°33′ E (Fig. 1). In this area, the relevés were distributed within the altitudinal range of the most representative alpine habitats, from approximately 2000 to 3000 m a.s.l. The climate is continental in the internal valleys, with low precipitation mainly concentrated in summer, and sub-Atlantic in the external belts, with slightly higher precipitation and lesser thermal excursions. Despite the scattered presence of carbonate outcrops, geological substrates are mainly metamorphic silicate rocks with gneiss and serpentinite, and alluvial and glacial deposits.

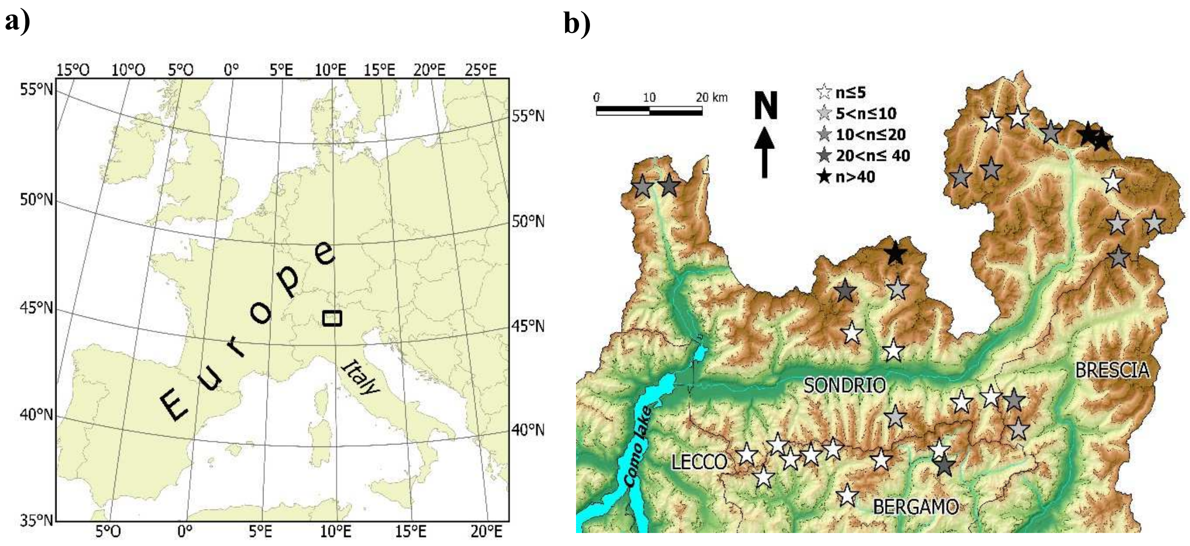

Figure 1: the administrative region of Lombardy (Northern Italy) delimited with a bold black line and **b** density of phytosociological relevés of alpine plant communities analyzed in this study within the georeferenced territorial units of the Lombardy regional database of habitats of Community Interest (Annex I of Directive 92/43/EEC) (Brusa et al. 2016)

2.2 Dataset and traits measurement

We based our analyses on phytosociological relevés representative of the main siliceous alpine plant communities of the study area, available in the Lombardy regional database of plant communities referring to Habitat types of EU Community interest (Annex I of Directive 92/43/EEC) (http://www.biodiversita.lombardia.it) (Appendix S1). We selected 382 relevés representative of three Habitat types on alpine silicate substrates (8110 'Siliceous scree of the montane to snow levels (*Androsacetalia alpinae* and *Galeopsetalia ladani*)', 6150 'Siliceous alpine and boreal grasslands', 4060 'Alpine and boreal heaths'), divided into six sub-habitats representative of a gradient of vegetation succession, from pioneer to stable and more highly structured plant communities (Table 1), according to Giacomini and Pignatti (1955). We adopted the classification of sub-habitats following the monitoring protocol of Habitat types of Community interest at the regional scale for region Lombardy (Brusa et al. 2017). Specifically, we identified the following sub-habitats: 8110-A 'Siliceous scree of the montane to snow levels of *Androsacion alpinae*', 6150-B 'Siliceous alpine and boreal grasslands of *Salicetalia herbaceae'*, 6150-A 'Siliceous alpine and boreal grasslands of *Caricetalia curvulae*' and 4060-A 'Alpine and Boreal heaths of *Loiseleurio-Vaccinion'*. We further divided sub-habitat 8110-A according to scree stability and the degree of plant colonization: instable screes with early colonization stages (8110-A1), and stable screes with relatively mature succession stages (8110-A2), following Caccianiga and Andreis (2004). Similarly, we divided sub-habitat 6150-A into two subsets depending on the intensity of the grazing pressure (mainly cattle pasture): low for communities dominated by *Carex curvula* (6150- A1) and high for communities dominated by *Festuca halleri* (6150-A2) (Giacomini and Pignatti 1955; Pierce et al. 2007b). For each plant species listed in the relevés, we collected data of LA, LDMC and SLA from authors' datasets, also available in TRY (Kattge et al. 2020, https://www.try-db.org/ see datasets n. 227, 228, 229, 371, 372 and related references). Additional data for missing species (27% of all species) were collected in the field, from samples of 5 to 15 fully expanded leaves from different individual adult plants, calculating LA, LDMC and SLA following the standardized methodological protocol for plant traits measurement (Perez- Harguindeguy et al. 2016).

Table 1: Sub-habitats (i.e., vegetation communities discernible within each Habitat type) analyzed in this study in relation to Habitat types classification (European Commission 2013) and CORINE biotopes (Commission of the European Community 1991). The range of the plot size is also reported (minimum and maximum). From top to bottom, the early and late successional stages, respectively. Legend: N, numbers of phytosociological relevés of each community

Habitat Code And Description	Sub-Habitat Code	Sub-Habitat	Corine Biotope	Syntaxa	N	Plot Size Range (M^2)
8110: siliceous scree of the montane to snow levels *Androsacetalia alpinae* and *Galeopsetalia ladani*)	8110-A1	Recent and/or disturbed moraines	61.111—Mountain sorrel screes	*Androsacetum alpinae*	98	6.25–500
	8110-A2	Stabilized moraines	61.112—Rock jasmine screes	*Oxyrietum digynae*		
			61.113—Alpine woodrush screes	*Luzuletum alpinopilosae*	42	6.25–500
				Saxifrago bryo-Poetum Alpinae		
	6150-B	Snowbeds	36.111 Alpine acid snow-patch communities	*Polytrichetum sexangularis*	69	1–100
				Salicetum herbaceae		
6150: siliceous alpine and boreal grasslands	6150-A1	Microthermal climax Grasslands	36.341—*Carex curvula* grasslands	*Caricetum curvulae*	89	1–100
	6150-A2	Microthermal grazed Grasslands	36.342—*Festuca halleri* grasslands	*Festucetum halleri*	51	1–100
4060: alpine and boreal heaths	4060-A	Summit heaths on wind-exposed ridges	31.41—Dwarf azalea and *Vaccinium* heaths	*Loiseleurieto-Cetrarietum*	33	4–100

2.3 Data analysis

We determined Grime's CSR (competitive, stress tolerant, ruderal) plant strategies using LA, SLA and LDMC, using the *StrateFy* CSR classification tool (Pierce et al. 2017). This classification method represents the extent of C-, S- and R-selection using trade-offs between traits (LA, SLA and LDMC), integrated and compared with trade-offs evident globally, hence we tested the functional identity of sub-habitats using both plant traits and CSR strategies. For each relevé, we estimated the community weighted mean (CWM, i.e., weighting the mean by the relative cover of each species in the relevé) of LA, LDMC and SLA, as well as of C-, S- and R-scores, using the R package '*FD*' (Lalibertè et al. 2014). The relative cover of each species in the relevé was obtained converting the ground cover indices (5 = 87.5%, 4 = 62.5%, 3 = 37.5%, 2 = 18.75%, 1 = 6.75%, + = 0.5%, r = 0.1%).

We applied the ANOVA with a Tukey post hoc comparison test to identify significant differences among the CWMs of plant traits and strategies scores between sub-habitats combining the functions '*aov*' and '*HSD.test*' of R packages '*stats*' and '*agricolae*', respectively (de Mendiburu 2019). Before running the ANOVA, we checked for normality of functional trait and plant strategy scores by means of the Shapiro–Wilk test and, accordingly, transformed LA and SLA by square root (sqrt[x]) and logarithmic (log[x + 1]) transformation, respectively, while no transformation was required for LDMC and strategy scores. Then, we identified and removed outlier values by applying the Rosner test to the CWM trait values and plant strategy scores within each sub-habitat, using the R package '*EnvStats*' (Millard 2013).

We used the R package '*composition*' (van den Boogaart et al. 2018) to visualize the ternary diagram of CSR strategies; compositional mean and variances of each sub-habitat were calculated using robust estimation. Then, we tested for differences in the CWMs of CSR ternary composition among sub-habitats applying the multivariate analysis of variance (using the 'MANOVA' function of the R package '*stats*'). For this purpose, we transformed the dataset of CSR scores by isometric log-ratio transformation (ILR), which reduced the ternary dataset into a two-dimensional image, in order to account for the compositional data structure (van de Boogaart and Tolosana-Delgado 2013; Dalle Fratte et al. 2019a).

To identify the relationship between species richness and total species cover (sum of all species relative cover per relevé) with the functional niches of dominance, stress and disturbance identifiable along the vegetation succession gradient, we plotted these vs. the CWMs of C-, S- and R-scores, considering all the relevés in all the sub-habitats. We then applied upper boundary regression, which is an extensively used method in ecological studies (Pierce 2014 and references therein) that fits a regression curve to the upper boundary of the dataset. This technique divides the continuous data on the x-axis into classes of equal range (known as bins) and fits a regression curve to the highest y values within each bin; in this case, the three highest values present within each 10% bins (of C-, S- and R-scores) were considered. Then, a Lorentzian 3-parameter regression curve was fitted by means of the '*nls*' function of the base R package '*stats*'. All the statistical analyses were computed with R software (R Core Team 2019).

3. Results

3.1 Plant traits

The CWMs of LA, LDMC and SLA showed significant differences between sub-habitats, and LDMC and SLA changed consistently along the succession gradient (Fig. 2 and Appendix S2). With regard to LA, grazed grasslands (sub-habitat 6150-A2) exhibited plants with the largest leaves, while dwarf-shrubs heathlands (sub-habitat 4060- A) included plants with the smallest. Leaves were smaller in less stable screes and snowbed sub-habitats (respectively, 8110-A1 and 6150-B), compared to the relatively stable screes and moraines sub-habitat (8110-A2). Both scree and moraine communities (sub-habitats 8110-A1 and 8110-A2) displayed the lowest leaf construction investment (lowest LDMC), while grasslands with low grazing pressure (sub-habitat 6150-A1) and heathlands (sub-habitat 4060-A) exhibited the highest leaf construction investment (highest LDMC). The snowbed sub-habitat (6150-B) exhibited intermediate values of LDMC, being significantly higher than screes and moraines and, simultaneously, lower than the other two grassland and heathland sub-habitats. LDMC was higher in communities with low grazing pressure (sub-habitat 6150-A1) and lower with intense grazing pressure (sub-habitat 6150-A2).

SLA exhibited the opposite pattern to LDMC along the succession gradient. Both scree and moraine communities (sub-habitats 8110-A1 and 8110-A2) exhibited the most acquisitive leaves (highest mean values of SLA), opposed to heathlands (sub-habitat 4060-A) which showed the most conservative leaves (lowest mean values of SLA). Snowbeds (6150-B) showed intermediate SLA values between pioneers and the other grassland communities. SLA was higher in communities that were subject to grazing (sub-habitat 6150- A2) and lower where the grazing pressure was less intense (sub-habitat 6150-A1).

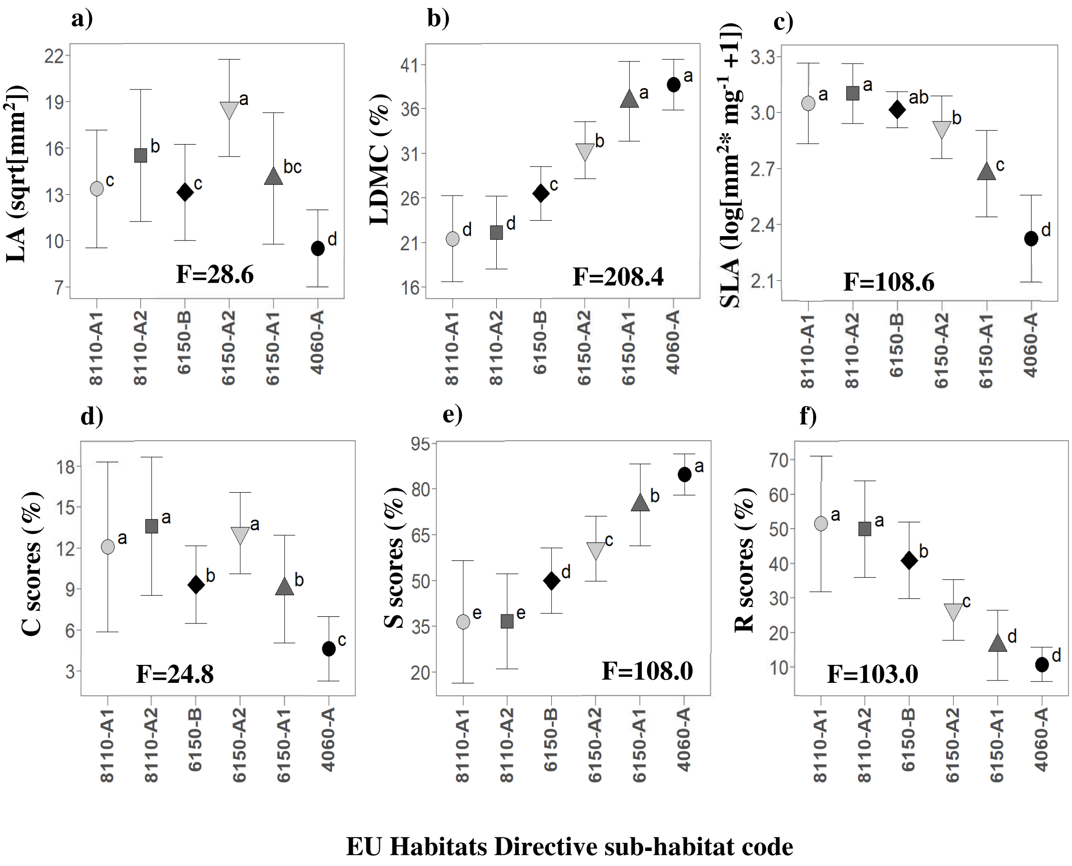

Figure 2: Mean values and standard deviation of community weighted means (CWMs) of a) leaf area (LA), b leaf dry matter content (LDMC), c specific leaf area (SLA), d C-strategy scores, e S-strategy scores, and f R-strategy scores for each sub-habitat (i.e., vegetation communities discernible within each Habitat type, see Table 1). Small letters indicate the results of the post hoc comparisons. Results of the ANOVA are also shown below each plot, indicating F-values; p values are always < 0.001.

3.2 Plant strategies

All the sub-habitats significantly differed in terms of CWM C-, S- and R-scores (Fig. 2 and Appendix S3); however, they showed a clear pattern only along S- and R- gradients. C-selection was the less discriminative; nonetheless, significant differences were still present. The most competitive communities (highest C-scores) were early succession stages and grazed grasslands, whereas summit heathlands of wind-exposed ridges (sub-habitat 4060-A) were the less competitive (lowest C-scores). Dwarf shrublands sub-habitats (4060-A) were also the most stress tolerant (highest S-scores), in contrast to scree and moraine communities (sub-habitats 8110-A1 and 8110- A2) which showed the lowest degree of S-selection. Grasslands with intense grazing pressure (sub-habitat 6150-A2) showed intermediate values of S-scores, being significantly higher than both scree and snowbed sub-habitats, and lower than grasslands with low grazing pressure and heathlands.

The pattern of R-selection opposed that for S-selection. Scree and moraine communities (sub-habitats 8110-A1, 8110-A2) were the most ruderal (highest values of R-scores) and heathlands (sub-habitat 4060-A) the less ruderal (lowest values of R-scores). Among grasslands, snowbeds showed the highest values of R-scores, while high grazing pressure (sub-habitat 6150-A2) was reflected in higher R-scores.

The sub-habitats differed significantly in terms of CSR ternary space occupation (Pillai = 0.61; F = 32.8; $p < 0.001$) and showed a robust variation along the succession gradient related to either unproductive niches (S-selection) or niches characterized by frequent disturbances to individuals (R-selection) (Fig. 3). Specifically, pioneer communities (sub-habitats 8110-A1 and 8110-A2) were placed toward the R-corner and displayed the highest variance, while at the other extreme of the succession gradient, heathlands (sub-habitat 4060-A) were located closer to the S corner and showed the lowest variance. Among grasslands, that with the lowest grazing pressure (sub-habitat 6150-A1) exhibited a functional signature similar to that of heathlands, while snowbeds (6150-B) exhibited a signature closer to that of scree and moraine communities and showed a lower variance (Appendix S4). Species richness and total species cover within the functional niches along the succession gradient Since the productive niche (C-corner) was scarcely represented within the analyzed communities, we fitted the regression curves only along the gradients of increasing CWMs of S- and R-strategy scores, which reflect the functional niches occupied by species in these habitats (Fig. 4). Species richness and total species cover displayed significant intermediate peaks along both S- and R-score gradients.

Along the S-selection gradient, or what can be interpreted as a ‘productivity niche gradient’, the highest values of species richness and total species cover were exhibited by alpine siliceous grasslands (EU Habitat type 6150), specifically by the grassland sub-habitat characterized by high grazing pressure (6150-A2), at intermediate values of S-selection (a range of between 40 and 60% CWM S-score). Species richness diminished toward both the lowest and the highest values of the S-selection gradient, following an almost unimodal symmetrical distribution.

Species richness and total species cover followed a positive unimodal asymmetrical distribution along the R-selection gradient. Indeed, they exhibited an intermediate peak at low values of the 'disturbance niches' gradient (a range of between 20 and 30% of R-scores), in correspondence of grasslands with high grazing pressure.

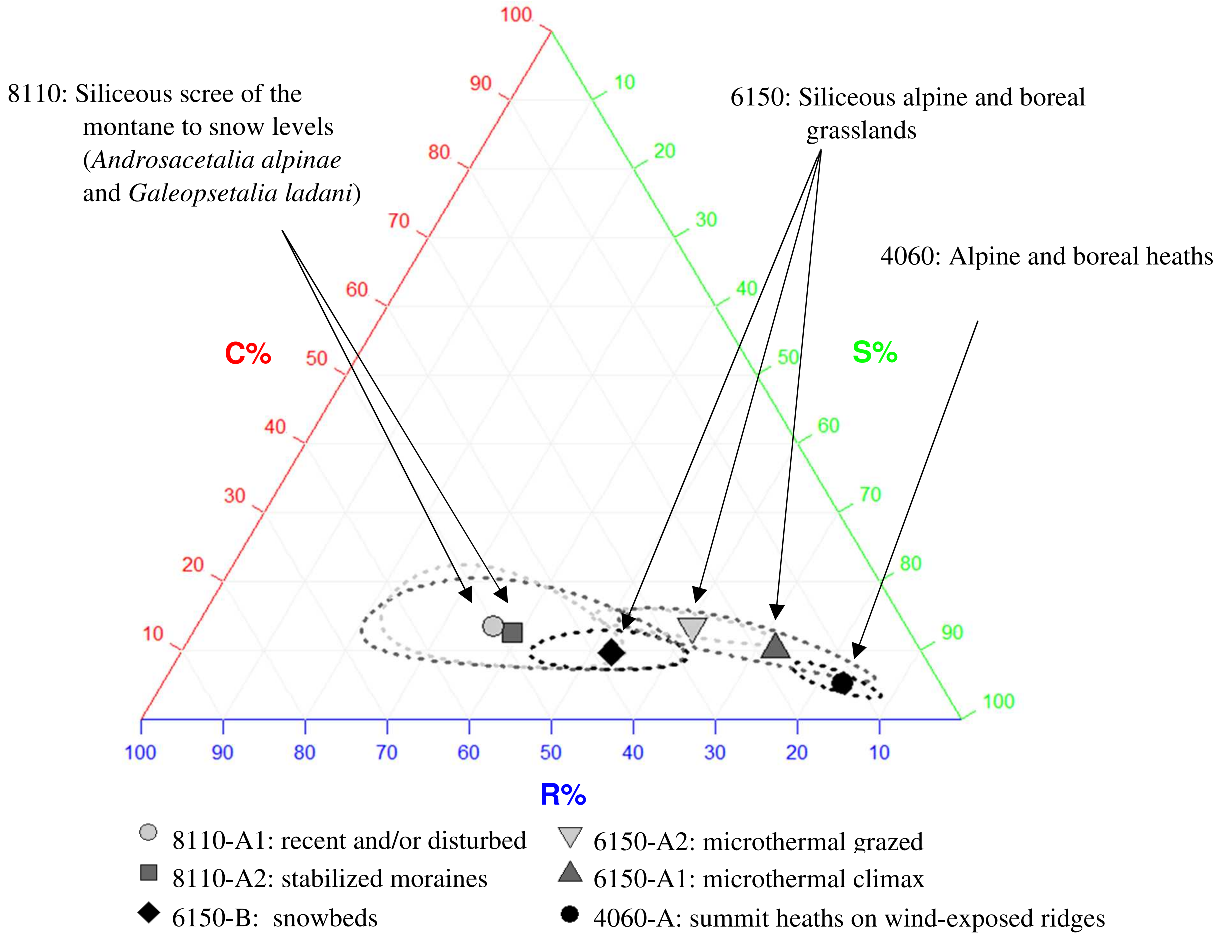

Figure 3: Community weighted means (CWMs) of each sub-habitat (i.e., vegetation communities discernible within each Habitat type, see Table 1) represented within the CSR strategy triangle displaying their means and variances.

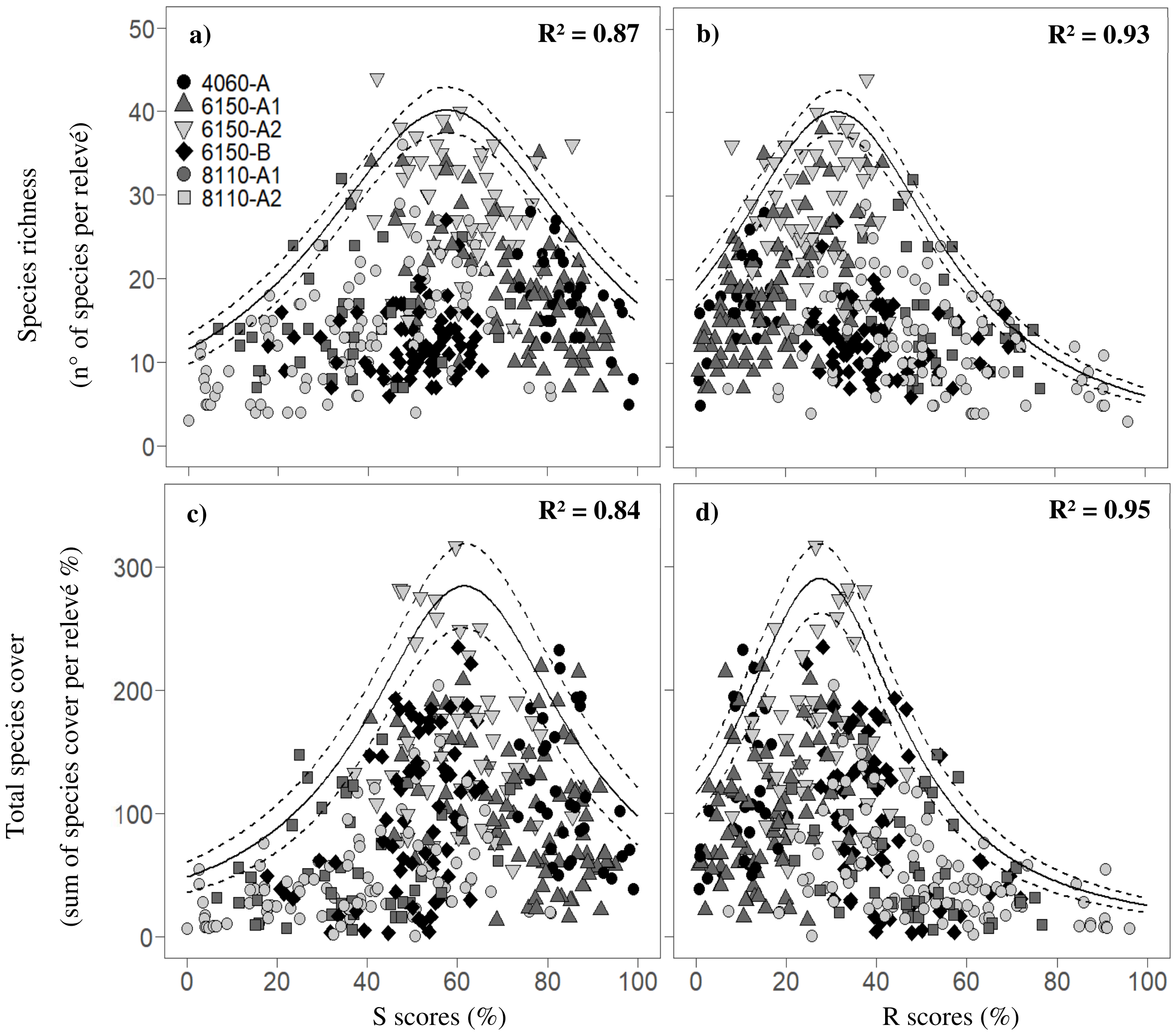

Figure 4: Upper boundary regression of species richness (**a**, **b**) and total species cover (**c**, **d**) of sub-habitats (i.e., vegetation communities discernible within each EU Habitat type, see Table 1) along the gradient of increasing community weighted means (CWMs) of S (**a**, **c**) and R (**b**, **d**) strategy scores. Lines represent the Lorentzian 3-parameter regression curve (continuous line) and its 95% confidence interval (dotted line). The significance of the fitting of the upper boundary regression curve is also shown in each plot, indicating R2 values; p values are always < 0.0001.

4. Discussion

This study provides an overview of how CWMs of plant traits and CSR ecological strategies are selected within alpine plant communities and indicate the type and range of realized niches occupied along a succession gradient. This regional-scale analysis of the community-level variation of plant functional traits and ecological strategies implements, and also validates, the recent observation of global-scale trade-offs that modulate trait combinations (plant height and resource acquisitiveness), not only between species (Díaz et al. 2016) but also between plant communities (Bruelheide et al. 2018). However, within alpine plant communities, size variation is scarcely evident, probably because these communities are characterized by a simple vertical structure with species belonging mostly to the herbaceous-shrubby layer (Thomas et al. 2020). Nonetheless, we found evidence that local-scale factors underpinning the vegetation succession gradient select trait combinations at the community level (Bruelheide et al. 2018), which are strongly reflected also within the framework of Grime's CSR strategy theory (Grime 2001; Grime and Pierce 2012).

The restriction of trait variability to a subset of those available in the regional species pool (i.e., functional convergence; Grime 2006) is typical of habitats of low temperatures (De Bello et al. 2013, Rosbakh et al. 2015; Wright et al. 2017; Thomas et al. 2020), such as those of high altitudes. However, the CWMs of traits relevant to the leaf economics spectrum (LDMC and SLA; Wright et al. 2004; Reich 2014) changed consistently with variations of the local environmental conditions underpinned by the succession gradient. Confirming previous observations (Grime 2001; Díaz et al. 2004; Gobbi et al. 2010; Li et al. 2017), communities from pioneer succession stages (screes, moraines and glacier forelands) are characterized by rapidly growing species (high SLA and low LDMC) that are replaced, as the succession goes on, by species with low relative growth rates and conservative–exploitative tendencies (low SLA and high LDMC).

However, such differences along succession gradients are almost never linked to just one environmental factor, but rather to their interactions. For instance, instable and stable scree sub-habitats showed similar CWMs of traits related to the leaf economics spectrum among them, almost comparable to snowbed communities (particularly SLA), highlighting the striking relationships between scree habitats and short growing seasons, because of the late snow melting in snowbeds, as also found in local-scale studies (Choler 2005). Considering that SLA is negatively related to leaf lifespan and positively related to relative growth rate (Reich 2014; Wright et al. 2004), to overcome this 'time constraint' for seasonal growth, these habitats select species with trait attributes that optimize carbon acquisition: fast-growing and low-cost leaves with short lifespan and high SLA (see also Kudo et al. 1999). Similar convergence also for LA due to late snow melting could be expected (Venn et al. 2011). However, among these three sub-habitats, LA increased with the greatest substrate stability (sub-habitat 8110-A2), possibly indicating a lower constrained allocation of resources favored by more stable substrates. We also observed the opposite trend in microthermal grasslands related to the effects of grazing pressure (Pierce et al. 2007b), which were characterized by fast-growing and acquisitive species. This may seem to contrast the expectation that communities exposed to high grazing pressure should develop resistance to herbivory by selecting species with tougher leaves (Pellissier et al. 2018). Despite this, grazing generates local disturbance due to cattle trampling and increases soil nutrient content and nutrient 'patchiness' (Peco et al. 2017), favoring exploitative species with greater colonization ability and faster growth rates (Niu et al. 2015).

These results are even more evident from the analyses of Grime's CSR plant strategies, which highlighted the ruderal to stress-tolerant gradient along the succession (Fig. 3). This strategy spectrum suggests that niche segregation and coexistence within alpine plant communities are mediated by a strong functional divergence with response to differential local stress and disturbance (see also Pierce et al. 2007b). The strong ruderal characteristics (rapid completion of the life cycle and selection of regenerative traits) exhibited by scree and snowbed sub-habitats are a response to their exposure to disturbance, which in alpine habitats is mainly represented by geomorphological and seasonal-climatic factors. Our results support the hypothesis that continued physical disturbance has a relevant role in the initial stages of primary succession (Matthews 1992; Caccianiga and Andreis 2004; Caccianiga et al. 2006; Ricotta et al. 2015; 2016; Pierce et al. 2017). Conversely, toward the late succession stages, climax grasslands and shrubland sub-habitats showed strong selection toward stress-tolerant characteristics, i.e., low productivity and conservative adaptations, typical of harsh niches. Generally, in the alpine context, seasonal variation and disturbance are marked and the period suitable for growth is often short, leading to a high survival risk for many species which must invest in defensive adaptations against such situations rather than in improving their competitive ability (Gobbi et al. 2010).

The functional convergence toward stress-tolerant strategies in mature communities demonstrates that alpine plant communities do not include productive niches that select for competitive strategies (Fig. 3). Competition is generally assumed to be more prominent at low elevations and at sites with low abiotic stress (Choler et al. 2001; Callaway et al. 2002), while it diminishes with altitude and relatively stressful physical conditions. However, the presence of this empty niche also suggests some possible future scenarios emerging from climate change. In particular, a warmer climate with longer growing season, as expected in the European Alps in future decades (Kovats et al. 2014; EEA 2017), could expose these habitats to the intrusion of new competitor species from lower altitudes or even exotic flora (Thuiller et al. 2008; Bjorkman et al. 2018; Dalle Fratte et al. 2019a, b). This scenario would result in shifting dominances of species within communities and in the formation of novel species assemblages and thus will modify ecological networks altering ecosystem processes (Walther 2010).

We found robust evidence at the regional scale that CSR theory is a powerful tool to investigate the functional niches available within communities (response to abiotic factors as mediated by CWMs of CSR strategies) and their structure (species richness and total species cover), refining similar observations that can be obtained using species plant traits trade-offs (Violle and Jiang 2009). Species richness and total species cover exhibited a unimodal distribution curve along both gradients of productivity (S-scores) and disturbance (R-scores) (Fig. 4), with microthermal grasslands mainly ordinated at the peak of the curve. In particular, grazed grasslands represented the sub-habitat with the highest species richness and total species cover. Indeed, although herbivory is a supplementary source of disturbance, it imposes strong selection on plant communities, suppressing potential dominant species and favoring diversity by allowing a larger number of subordinate species to coexist (Pierce et al. 2007a, b; Cerabolini et al. 2016).

It is widely recognized that the pattern between community diversity and disturbance is well predicted by a unimodal curve, according to the humped-back model (Grime 1973). Here, we confirm that in alpine context this pattern is highly consistent also in terms of the total species cover; in fact, its maximum corresponds with that of species richness along the same gradient. This may seem in conflict with the humped-back curve of biodiversity and biomass (e.g., Pierce 2014; Fraser et al. 2015; Cerabolini et al. 2016). However, as the competitive strategy is not represented in alpine ecosystems, the right-hand flank of the humped-back model is not well represented (the side related to the competitive niche, Grime and Pierce 2012) and high species richness corresponds to the highest biomass production. While an increase in stress or disturbance drives a homogeneous selection toward high niche differentiation, their weakening leads to differential increase in species richness and biomass along Sand R-scores (respectively, symmetrical and positive asymmetrically distributed). Distinct processes thus operate along the two gradients, specifically, the faster increase in species diversity and biomass with decreasing stress agrees with the stress gradient hypothesis (Maestre et al. 2009; Malkinson and Tielbörger 2010), which suggests that facilitation is more common in conditions of high abiotic stress.

5. Conclusions

This study demonstrated that alpine communities are strongly selected in terms of the economics spectrum, but the stable, productive niche typical of C-selected strategies is scarcely represented, suggesting that trade-offs between communities at the global scale can experience adaptions at the regional scale caused by local environmental conditions. Moreover, Grime's CSR strategies allowed a more precise functional interpretation of alpine vegetation along the succession gradient compared to single plant traits, allowing the identification of realized functional niches within alpine communities. Our results proved these relationships to be robust over a regional scale, and not only at a local scale as for previous studies, thus that they provide a legitimate general representation of siliceous alpine vegetation.

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Appendix S2: Community Weighted Means (CWMs) of leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA) (mean ± standard deviations) within all the analyzed sub-habitats (i.e. vegetation communities discernible within each EU Habitat type). From top to bottom, the early and late successional stages respectively. Values for LA and SLA refer to transformed data, respectively sqrt(x) and log(x+1).

SUB-HABITAT	CWM_LA ± sd	CWM_LDMC ± sd	CWM_SLA ± sd
Recent and/or disturbed moraines (8110-A1)	13.3 ± 3.8	21.4 ± 4.8	3.0 ± 0.2
Stabilized moraines (8110-A2)	15.5 ± 4.3	22.1 ± 4.1	3.1 ± 0.2
Snowbeds (6150-B)	13.1 ± 3.1	26.5 ± 3.0	3.0 ± 0.1
Microthermal grazed grasslands (6150-A2)	14.0 ± 4.2	36.8 ± 4.5	2.7 ± 0.2
Microthermal climax grasslands (6150-A1)	18.6 ± 3.1	31.4 ± 3.2	2.9 ± 0.2
Summit heaths on wind-exposed ridges (4060-A)	9.5 ± 2.5	38.7 ± 2.8	2.3 ± 0.2

Appendix S3: Community Weighted Means (CWMs) of C - competitive, S – stress-tolerant, and R - ruderal strategies scores (mean ± standard deviations) within all the analyzed sub-habitats (i.e. vegetation communities discernible within each EU Habitat type). From top to bottom, the early and late successional stages respectively.

SUB-HABITAT	CWM_C ± sd	CWM_S ± sd	CWM_R ± sd
Recent and/or disturbed moraines (8110-A1)	12.1 ± 6.3	36.5 ± 20.1	51.4 ± 19.6
Stabilized moraines (8110-A2)	13.6 ± 5.1	36.6 ± 15.6	49.8 ± 13.9
Snowbeds (6150-B)	9.3 ± 2.8	49.9 ± 10.8	40.8 ± 11.0
Microthermal grazed grasslands (6150-A2)	13.1 ± 3.0	60.4 ± 10.7	26.5 ± 8.8
Microthermal climax grasslands (6150-A1)	9.0 ± 3.9	74.8 ± 13.4	16.3 ± 10.2
Summit heaths on wind-exposed ridges (4060-A)	4.6 ± 2.4	84.7 ± 6.8	10.7 ± 5.0

Appendix S4: CSR compositional Community Weighted Means (CWMs) (mean ± variance) within all the analyzed sub-habitats (i.e. vegetation communities discernible within each EU Habitat type). From top to bottom, the early and late successional stages respectively.

SUB-HABITAT	CWM (C : S : R ± Var)
Recent and/or disturbed moraines (8110-A1)	12.6 : 39.0 : 48.4 ± 72.0
Stabilized moraines (8110-A2)	13.3 : 36.3 : 50.4 ± 56.2
Snowbeds (6150-B)	9.7 : 52.5 : 37.8 ± 16.8
Microthermal grazed grasslands (6150-A2)	13.5 : 60.5 : 26.0 ± 20.1
Microthermal climax grasslands (6150-A1)	10.1 : 72.4 : 17.6 ± 61.8
Summit heaths on wind-exposed ridges (4060-A)	5.1 : 83.1 : 11.8 ± 11.1

Towards a Functional Phytosociology: the functional ecology of woody diagnostic species of European vegetation classes

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Submitted to *Journal of Vegetation Science* on 23-10-2020

Abstract

Vegetation is often classified using a phytosociological approach, which aims to define floristically coherent units corresponding to particular ecological contexts through the identification of diagnostic species. Species- and community-environment relations are key elements of phytosociology and are potentially regulated by plant functional traits that underpin plant survival and plant community composition, although an explicit link between functional ecology and phytosociology is still lacking. Here we asked whether the functional analysis of sets of woody diagnostic species of vegetation classes can identify a correspondence between their functional properties and the physiognomy and ecology of the vegetation they represent, and also which are the principal environmental factors that determine plant assemblages, adaptions and responses. We considered 221 woody diagnostic species (including alien neophytes) of 21 European phytosociological classes of woody vegetation; we measured leaf (leaf area, specific leaf area, leaf dry matter content, carbon to nitrogen ration, leaf nitrogen content) and whole plant (canopy height, seed mass) functional traits, and calculated Competitor, Stress-tolerant, Ruderal (CSR) strategies. We identified traits multidimensional functional space by Principal Component Analysis and tested for differences among species and classes either by multivariate or univariate phylogenetic analysis of variance. Species and classes showed a functional pattern coherent with their physiognomy and ecology, following that of the global spectrum of plant form and function. Within the CSR ternary functional space, the sets of woody diagnostic species mainly aligned along the S-C gradient: deciduous forests and neophyte-dominated classes displayed the highest values of C- scores, whereas evergreen-dominated classes were mostly stress tolerant. The ruderal strategy was not much represented. We demonstrated that functional traits and CSR strategies reliably indicate plant-environment relations underpinned by the phytosociological classification method, suggesting a solid link between phytosociology and functional ecology, at least for woody vegetation classes.

Keywords:

CSR, adaptive strategies, plant traits, functional ecology, IAS, neophytes, phytosociology, global spectrum, forests, shrublands, heathlands, *syntaxa*.

1. Introduction

Phytosociology is one of the most well-known and widely applied approaches to vegetation classification. This method categorizes vegetation into floristically coherent units called *syntaxa* (Mucina *et al.*, 2016) by the analysis of lists of species recorded in vegetation plots (Chytrý and Thichý, 2003; De Caceres *et al.*, 2015). Of particular relevance is the use of diagnostic species that occur in a single vegetation unit (character species) or in a few vegetation units (differential species), and that indicate a specific *syntaxon,* separating it from all the others (Chytrý *et al.*, 2002a). Diagnostic species can be statistically determined by measuring 'fidelity', a measure of species concentration in a given vegetation unit, and their corresponding scarcity or absence in other units; species exceeding a fidelity threshold are considered diagnostic (Chytrý *et al.,* 2002b). *Syntaxa* are included in a floristically defined hierarchical classification system (e.g. Rivas-Martínez *et al.,* 2001; Biondi *et al.,* 2014; Mucina *et al.*, 2016) based on the presence of common groups of diagnostic species that allow the identification of higher hierarchical levels, and each rank level is characterized by its own set of diagnostic species (Biondi, 2011). The basic unit of phytosociology is the 'association', which is defined as '*a vegetal grouping more or less stable and in equilibrium with the environment, characterized by a particular floristic composition, in which some exclusive or almost exclusive elements (characteristic species) reveal with their presence a particular and autonomous ecology*' (Braun-Blanquet, 1928). The highest and most comprehensive rank is the 'class', which also carries ecological information since it is associated to a given biogeographic context and reflects the niches of the species from which is composed (Pignatti *et al.*, 1995; Biondi *et al.*, 2014).

Generally, phytosociology recognizes the relationships between plant communities and environmental factors (Braun-Blanquet, 1964), as its principal goal is the definition of vegetation types based on their floristic composition in relation to given ecological contexts. The study of species- and community-environment relations is key to the functional interpretation of plant communities (Dengler, 2008; Pott, 2011), therefore concepts related to vegetation functions are implicitly included within the phytosociological approach but remain scarcely investigated. Indeed, the field of functional ecology organizes species according to their adaptive traits, both spectra of trait variability and by delimiting functionally homogeneous groups, i.e. functional types, defined as '*sets of species showing similar responses to the environment and similar effects on ecosystem functioning*' (Smith *et al.*, 1992; Díaz and Cabido, 1997; 2001; Lavorel and Garnier, 2002). These reflect plant assemblages responding coherently to abiotic and biotic environmental factors. A trait-based approach is known to provide quantitative and predictive insights into the general mechanics of plant communities and ecosystem functioning, in contrast to descriptive approaches based on species identity only (Díaz and Cabido, 2001; Dubuis *et al.*, 2013; Adler *et al.,* 2013).

The adoption of functional traits has made it possible to define the functional spaces occupied by species. The global spectrum of plants form and function (Díaz *et al*., 2016), for instance, shows that the trade-offs between six key traits of vascular plants (linked to survival, growth and reproduction) are determined by two main axes of adaption: the size of plants and their parts and resource economics (i.e. resource acquisition and conservation, typically measured as 'leaf economics') (Wright *et al*., 2004; Reich, 2014). Another related space is that defined by CSR plant strategies (Grime, 1974; 1979; 2006) where species are ordinated along axes based on trait values reflecting their response to the main ecological drivers underlying vegetation processes, such as the interactions between competition, stress and disturbance (Pierce *et al*., 2017). While these constraints describe species adaptations and strategies, many studies have analyzed community-level trait composition (e.g. McGill *et al*., 2006; Garnier *et al*., 2016) which is necessary to gain further insight into environmental drivers. Bruelheide *et al.* (2018) have also recently defined the functional space of plant communities at the global extent, starting from plot-level trait composition. Nonetheless, functional analyses of plant communities as identified by the phytosociological classification system have been attempted only at low rank levels, such as association (e.g. Poldini *et al*., 2011; Zanzottera *et al*., 2020) and order (Macedo *et al*., 2010), whereas at higher ranks the functional connotation of *syntaxa* has rarely been assessed (Illa *et al*., 2006; Macedo *et al*., 2010), especially on a wider range of communities. The distribution of functional traits in high rank plant assemblages (i.e. classes) across a broad range of different habitats could provide an improved characterization of plant communities with respect to the traditional phytosociological system determined by the occurrence or abundance of species. For instance, recent evidence suggests that a trait-based approach could use diagnostic species as indicators of plant community functioning (Ricotta *et al*., 2015; 2020).

These goals could be achieved by the functional analysis of diagnostic species representative of classes, which would provide better understanding of their relationships with environmental factors. This in turn would improve understanding of the impact of the main environmental drivers that shape plant communities, such as stress, disturbance (also derived from land use change), and competition (which can also be related to invasion by alien species). Indeed, naturalized alien species nowadays are part of the natural vegetation of Europe to the point that several alien-dominated *syntaxa* have been recognized (e.g. Viciani *et al*., 2020), and they are also often included within the list of diagnostic species of *syntaxa* of spontaneous vegetation (Mucina *et al.,* 2016). The spread and the impacts of alien species are context-dependent, particularly within sensitive ecosystems (Lamarque *et al*., 2011; Pyšek *et al*., 2012), and their comparison across habitats and regions is still much needed (Rabitsch *et al*., 2016; Dick *et al*., 2017), Thus, it is necessary to understand their functional roles in natural assemblages and which environments are potentially prone to invasion. Clarifying the link between functional ecology and phytosociology could also help achieve this goal.

In this study we analysed 221 woody diagnostic species, including alien species, in terms of their functional traits. The study species are representative of 21 European woody vegetation classes, including anthropogenic ones, and range from low shrub to forest formations and include mediterranean to boreal/alpine climatic belts. We selected woody species because they best represent the structure of the community they characterize. Indeed, the set of diagnostic (or characteristic) species used to describe *syntaxa* are also defined according to the dominant species in terms of vegetation structure. Such species are one of the most evident characteristics of a plant community and are linked to its physiognomy, and consequently to its structure, providing the initial elements for the recognition of the *syntaxa* (Biondi, 2011). Therefore, an analysis of the functional traits trade-offs for woody diagnostic species of classes can be expected to play an important role in clarifying existing ecosystem dynamics through a more detailed analysis of form, function and plant–environment interactions (Gillison, 2013).

We aimed to assess how woody species, diagnostic of phytosociological classes, are ordinated within the functional space defined by resource economics and size traits (and thus the global spectrum of plant form and function and CSR strategies) and relate this to the structure and physiognomy of the classes they represent. We addressed the hypothesis that the functional space occupied by woody diagnostic species is directly indicative of the main environmental drivers that shape plant communities. Specifically, we paid particular attention to processes and potential threats to communities, such as invasion by alien species.

2. Methods

2.1 Functional traits measurement and dataset creation

For this study we selected a sample of species (n = 221) representative of 21 classes of European woody vegetation, ranging from mediterranean to boreal/alpine bioclimatic zones, including vegetations dominated by both native species (including archaeophytes, i.e. alien species introduced in Europe before year 1.500 A.D., Pyšek *et al.*, 2003) and neophyte species (i.e. those introduced in Europe after year 1.500 A.D., Pyšek *et al.*, 2003) (Table 1). We assigned each species to one or more corresponding classes according to the list of diagnostic species of European vegetation (EuroVeg checklist; Mucina *et al.*, 2016; Appendix S1). We considered only classes for which functional traits were available for at least five species. Our dataset also included neophytes (n = 15; see Appendix

S1), since these are recognised as diagnostic species for some European vegetation classes (Mucina *et al.*, 2016).

To take into account the two main axes of variation of the global spectrum of plant form and function (Diaz *et al.*, 2016), for each species we selected three functional traits related to the size of the plant and its organs, Leaf Area (LA), Canopy Height (CANH) and Seed Mass (SM), and two functional traits known to be highly representative of leaf economics, Specific Leaf Area (SLA) and Leaf Nitrogen Content (LNC). We also added Carbon to Nitrogen ratio (C/N) and Leaf Dry Matter Content (LDMC), in order to account for litter decomposition and carbon balance (Freschet *et al.*, 2010; Pierce *et al.,* 2007). Moreover, LA, LDMC and SLA are also the traits used to calculate Grime's CSR (Competitive – Stress Tolerant – Ruderal) plant strategies using the *StrateFy* classification tool (Pierce *et al.*, 2017), which was used in the present study to calculate CSR scores (for methodological details see also Pierce *et al.* 2013).

Trait data were collected from the Authors' datasets (Cerabolini *et al.*, 2010), also available as part of the TRY global functional trait database (datasets 227 and 228; Kattge *et al.*, 2020), and from the LIFTH database (Dalle Fratte *et al.*, 2020), except for CANH and SM, which were derived respectively from Pignatti (1982) and the Kew Garden Seed Information Database (SID, https://data.kew.org/sid). Methodological details of processing procedures are also reported in Dalle Fratte *et al.* (2020). Species were additionally classified with respect to their growth form (sub-shrubs, shrubs, trees and climbers) and leaf lifespan (deciduous or evergreen) according to Pignatti (1982). Species were sampled at 69 sites, covering an area of approximately 65.000 Km^2 from sea level to 2650 m a.s.l. and a wide range of climatic regimes: from continental middle-European in lowland areas, to a mediterranean regime near the coastline, and a cold oceanic alpine climate in the mountains. Substrates at sample sites include both silicate and carbonate rocks of different derivation, with high local variability. The entire dataset is available in Appendix S1.

2.2 Data analysis

All the statistical analyses were computed with R software (R Core Team, 2020). We applied hierarchical (taxonomy: family and genus) based gap filling, using the function "GapFilling" of the *BHPMF* package (Fazayeli *et al.*, 2017) to complete missing data for LNC (n =3), C/N (n = 3) and SM (n = 10). Gap filling was applied using all data from the Authors' dataset described above (n = 1746) in order to include a greater number of *taxa*, allowing greater prediction accuracy. We then normalized data by logarithmic (LA, CANH, C/N and SM) or square root (SLA and LNC) transformation according to the outcome of Shapiro-Wilks tests; no transformation was required for LDMC data, which was found to exhibit a normal distribution.

To confirm whether the plant global spectrum of plant form and function was reflected within the woody species of our dataset, we performed a Principal Component Analysis (PCA), followed by varimax rotation, on functional traits data using the "principal" function in the R-package *psych* (Revelle, 2017). After determining the significant components of the PCA (i.e. those that explained most of the dataset total variance), we tested for differences in the spatial arrangement of growth forms and leaf lifespans within the multidimensional space, by means of multivariate analysis of variance (MANOVA). We then applied one-way analysis of variance (ANOVA), followed by post-hoc pairwise comparison, to identify significant differences along each significant component of the PCA. In order to account for phylogenetic correlation, we applied phylogenetic MANOVA by means of the "aov.phylo" function of the R-package *geiger* (Harmon *et al.*, 2008), and phylogenetic ANOVA with *post-hoc* tests using respectively the functions "phylANOVA" and "multcompLetters" of R-packages *phytools* (Revelle, 2012) and *multcompView* (Graves *et al.*, 2015).

We then investigated whether the functional spectrum of the classes differed from each other in a coherent way (i.e. respecting the physiognomy of the classes), through hierarchical clustering on principal components using the function "HCPC" of the R-package *FactoMineR* (Le *et al.*, 2008). The consistency of gained clusters was checked within the ternary space determined by CSR strategies by means of phylogenetic MANOVA. To account for the compositional structure of the CSR data we first transformed the dataset of CSR scores with Isometric Log-Ratio Transformation, which reduced the ternary dataset to a two-dimension image (see Dalle Fratte *et al.* (2019) and references therein). We also compared the C-, S-, and R-scores through phylogenetic ANOVA followed by *post-hoc* comparison. We used the R-package *ggtern* (Hamilton and Ferry, 2018) to visualize the ternary plot of CSR strategies. Finally, we compared native and neophyte species along both significant components of the PCA as well as C-, S- and R-scores by means of phylogenetic ANOVA.

2.3 Phylogenetic tree

We built a phylogenetic tree for the study species using the megaphylogeny tree implemented in the package *V.PhyloMaker* (Jin and Qian, 2019). We adopted their 'Scenario 1' to solve the phylogeny at the species level in our dataset, since it is the most cautious and avoids random solutions by adding genera or species as basal polytomies within families or genera. Before processing the phylogenetic tree, we standardized names of every taxon (family, genus and species) in our dataset according to The Plant List (http://www.theplantlist.org) using the R package *Taxonstand* (Cayuela *et al.*, 2012).

Table 1: List of the 21 classes of European woody vegetation selected for the analysis and their corresponding abbreviation code according to the EuroVeg checklist (Mucina et al. 2016). The number of diagnostic species belonging to each class (n) is reported.

Code	Class	Decription	Vegetation type	n
AC (LOI)	Loiseleurio procumbentis-Vaccinietea	Arctic-boreal tundra scrub and relict alpine acidophilous dwarf-heath mountain tundra of Eurasia and North America	vegetation of the arctic zone	10
BA (PIC)	Vaccinio-Piceetea	Holarctic coniferous and boreo-subarctic birch forests on oligotrophic and leached soils in the boreal zone and at high-altitudes of mountains in the nemoral zone of Eurasia	vegetation of the boreal zone	19
CA (FAG)	Carpino-Fagetea sylvaticae	Mesic deciduous and mixed forests of temperate Europe, Anatolia, the Caucasus and Southern Siberia	vegetation of the nemoral forest zone	40
CB (PUB)	Quercetea pubescentis	Oak, mixed deciduous and conifer woods of warm regions in the cool-temperate nemoral zone of Central and Southern Europe and in the supramediterranean belt of the Mediterranean, Asia Minor and Middle East	vegetation of the nemoral forest zone	34
CC (QUE)	Quercetea robori-petraeae	Acidophilous oak and oak-birch forests on nutrient-poor soils of Europe	vegetation of the nemoral forest zone	10
CD (RHA)	Crataego-Prunetea	Scrub and mantle vegetation seral or marginal to broad-leaved forests in the nemoral zone and the submediterranean regions of Europe	vegetation of the nemoral forest zone	41
CE (LON)	Lonicero-Rubetea plicati	Acidophilous scrub and hedges of forest edges and clearings on dry sandy nutrient-poor minerotrophic soils of Western Europe	vegetation of the nemoral forest zone	6
CF (ROB)	Robinietea	Seral forest-clearing and anthropogenic successional scrub and thickets on nutrient-rich soils of temperate Europe	vegetation of the nemoral forest zone	15
CH (ULI)	Calluno-Ulicetea	Heath on acidic nutrient-poor soils in the lowland to montane belts of the temperate and boreal zones of Europe	vegetation of the nemoral forest zone	8
CN (SAB)	Junipero-Pinetea sylvestris	Relict oromediterranean and submediterranean orotemperate dry pine forests, juniper woods and related scrub of the Mediterranean	vegetation of the nemoral forest zone	5
CO (ERI)	Erico-Pinetea	Relict pine forests and related scrub on calcareous and ultramafic substrates of the Balkans, the Alps, the Carpathians and Crimea	vegetation of the nemoral forest zone	14
CP (MUG)	Roso pendulinae-Pinetea mugo	Pine krummholz in the subalpine belts of the nemoral mountain ranges of Europe	vegetation of the nemoral forest zone	7
CQ (RHO)	Rhododendro hirsuti-Ericetea carneae	Supramontane to subalpine low heath on calcareous skeletal soils, rocky outcrops, lapiés and boulders of the Alps, the Apennines and the Dinarides	vegetation of the nemoral forest zone	8
CR (VIR)	Betulo carpaticae-Alnetea viridis	Subalpine and subarctic herb-rich alder and willow scrub and krummholz of the Alps, the Carpathians, the Hercynicum, the Balkans, the Caucasus, Northern Europe and Greenland	vegetation of the nemoral forest zone	12
FA (QUI)	Quercetea ilicis	Thermo-mesomediterranean pine and oak forests and associated macchia of the Mediterranean	vegetation of the mediterranean zone	29
FB (ROS)	Ononido-Rosmarinetea	Mediterranean scrub (tomillar, espleguer, romeral, garrigue, phrygana, batha) on base-rich substrates	vegetation of the mediterranean zone	17
FC (LAV)	Cisto-Lavanduletea	Mediterranean scrub (jaral, matorral, garrigue, phrygana) on acidic siliceous and ultramafic substrates	vegetation of the mediterranean zone	6
HA (POP)	Alno glutinosae-Populetea albae	Riparian gallery forests of the Eurosiberian and Mediterranean regions	alluvial forests and scrubs	23
HB (PUR)	Salicetea purpureae	Willow and tamarisk scrub and low open forests of riparian habitats in the temperate to arctic zones of Europe and Greenland	alluvial forests and scrubs	7
IA (ALN)	Alnetea glutinosae	European mesotrophic regularly flooded alder carr and birch wooded mires	swamp forests and scrubs	6
PB (OXY)	Oxycocco-Sphagnetea	Dwarf-shrub, sedge and peat-moss vegetation of the Holarctic ombrotrophic bogs and wet heath on extremely acidic soils	vegetation of bogs and fens	5

3. Results

The first two principal components (PC1 and PC2) of the PCA together explained a large amount (71 %) of the dataset total variance (respectively, 42 % and 29 %). Functional traits displayed a specific pattern along the PCA axes: PC1 correlated positively with SLA and LNC and negatively with LDMC and C/N, while PC2 correlated positively with LA, CANH and SM (Fig. 1a, Table 2). This disposition reflected variation from conservative to acquisitive leaf traits along PC1 (thus representing the leaf economics spectrum), and the increase in size dimensions of plants and their organs along PC2. Moreover, both leaf lifespan (Wilks = 0.59, approx.-F = 74.4, phylog. corrected $p < 0.001$) and growth form categories (Wilks = 0.37, approx.-F = 46.1, phylog. corrected $p < 0.001$) displaced significantly differently in the multidimensional space of the PCA, mirroring functional trait variation. Leaf lifespan showed significant differences only along the 'PC1economics' axis, with deciduous species relatively acquisitive compared to evergreen species, while growth form variation was related to the 'PC2-size' axis, with trees exhibiting higher values opposed to sub-shrubs (Appendix S2).

The two dimensions of plant form and function were strongly reflected also among classes. Both PC1-economics ($eta^2 = 0.72$, $p < 0.001$) and PC2-size ($eta^2 = 0.93$, $p < 0.001$) contributed significantly in the delimitation of four groups of classes: evergreen heaths and scrubs, deciduous shrubs (meso-hygrophilous formations of avalanche slopes and riparian habitats), evergreen forests (including both coniferous and sclerophyllous formations), and deciduous forests (Appendix S3). Specifically, along the PC1-economics axis, deciduous forests and, even more so, deciduous scrubs, showed more acquisitive characteristics compared to heaths and scrubs as well as evergreen forest, with the class *Robinieta* (CF.ROB) being the most acquisitive, as opposed to the most conservative *Junipero-Pinetea sylvestris* (CN.SAB). Along the PC2-size axis, both deciduous and evergreen forests exhibited higher values compared to heaths and scrubs as well as deciduous shrubs. Specifically, deciduous forests showed the highest mean values with the class *Carpino-Fagetea sylvaticae* (CA.FAG) and *Alno glutinosae-Populetea albae* (HA.POP), as opposed to the lowest mean values of the heath and scrub classes *Ononido-Rosmarinetea* (FB.ROS) and *Calluno-Ulicetea* (CH.ULI) (Appendix S4).

Considering the ternary CSR plant strategy space (Fig. 2), the mean values of classes were ordinated essentially along a gradient from stress-tolerant (S) to competitive (C) strategies. Deciduous forests exhibited significantly higher mean values of C-scores than evergreen forests as well as heath and scrub classes, which conversely had significantly higher mean values of S-scores. Specifically, the class *Junipero-Pinetea sylvestris* (CN.SAB) was the most stress-tolerant, as opposed to the most competitive (and neophyte-dominated) class *Robinietea* (CF.ROB). We did not find differences among these groups of classes regarding their R-scores (Appendix S5), despite a slight tendency of meso-hygrophilous formations towards ruderal strategies.

Neophyte species displayed both higher acquisitive characteristics and larger size of their organs (i.e. higher mean value along both PC1-economics and PC2-size), and simultaneously showed a greater mean value of C-scores and lower of S-scores. We did not find significant differences of neophytes in terms of R-scores (Fig. 1b, Fig. 2 and Appendix S6).

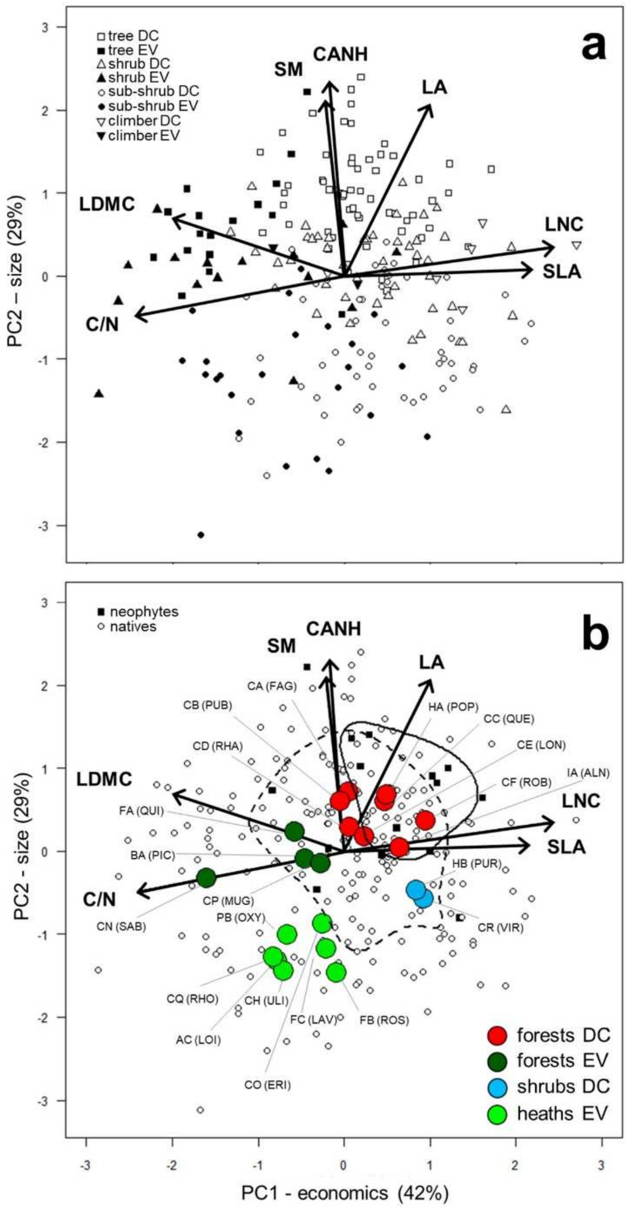

Figure 1: Principal Component Analysis on trait values of the 221 woody diagnostic species considered for the analysis grouped by growth form and leaf life span (deciduous = DC, evergreen = EV) (a), and neophyte vs. native species (b). Mean values of the classes (full names are reported in Table 1) are also shown (b), grouped by the cluster they belong to (Figure S4). Lines represent the 50th percentile of the distribution of neophyte (solid line) and native species (dashed line). Legend: CANH = canopy height, C/N = carbon to nitrogen ratio, LA = leaf area, LDMC = leaf dry matter content, LNC = leaf nitrogen content, SLA = specific leaf area, SM = seed mass.

Table 2: Pearson's correlation coefficients (r) of trait values with the first two axes of the Principal Component Analysis (PC1 and PC2) for the 221 study species. Bold values are highly correlated (r > ± 0.5) and statistically significant ($p \leq 0.01$) (critical value of r = 0.17, df = 219). Legend: CANH = canopy height, C/N = carbon to nitrogen ratio, LA = leaf area, LDMC = leaf dry matter content, LNC = leaf nitrogen content, SLA = specific leaf area, SM = seed mass.

Plant trait	PC1	PC2
C/N	**-0.90**	-0.18
CANH	-0.06	**0.86**
LA	0.37	**0.76**
LDMC	**-0.74**	0.25
LNC	**0.90**	0.13
SLA	**0.80**	0.03
SM	-0.08	**0.78**

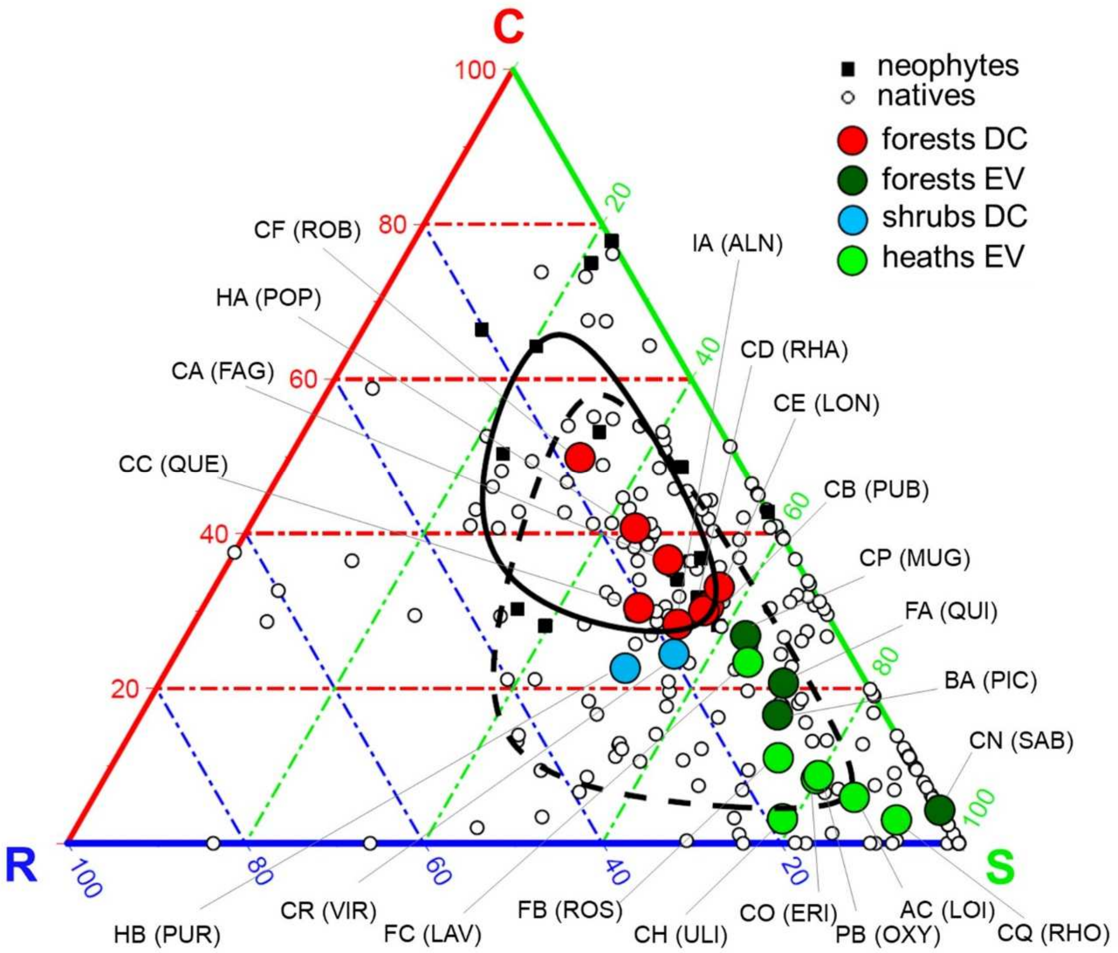

Figure 2: Ternary visualization of Grime's CSR (Competitive, Stress-tolerant, Ruderal) plant strategies of the 221 woody diagnostic species selected for the analysis, showing neophyte vs. native species, and mean values of classes (full names are reported in Table 1), grouped by the cluster they belong to (see Figure S4). Lines represent the 50th percentile of the distribution of neophyte (solid line) and native species (dashed line). Legend: DC = deciduous, EV = evergreen.

4. Discussion

Our results demonstrate that the functional characteristics of woody species, representative of a specific subset of diagnostic species of phytosociological classes of European woody vegetation, can effectively embody the physiognomy and the ecology of the vegetation they represent, supporting our hypothesis. Trade-offs among the traits we considered were well captured by the first two dimensions of principal component analysis (PCA) (Fig. 1a), showing that variation in resource economics and size are common to both studies of global functional ecology (e.g. Westoby *et al.,* 2002; Garnier *et al*., 2004; Fyllas *et al*., 2020) and to vegetation units resolved by phytosociology. We determined that phytosociological classification was associated with two main axes of variation: the first represented the leaf economics axis (Wright *et al.,* 2004; Reich, 2014), going from communities with resource-acquisitive characteristics, e.g. classes *Alnetea glutinosae* (IA.ALN) and *Betulo carpaticae-Alnetea viridis* (CR.VIR), to those with resource-conservative features typical of evergreen classes, such as *Junipero-Pinetea silvestris* (CN.SAB). The second axis corresponded to size variation, going from short-stature and light-seeded communities typical of heath and scrub classes, e.g. *Ononido-Rosmarinetea* (FB.ROS) and *Calluno-Ulicetea* (CH.ULI), to tall-stature and heavy-seeded communities typical of forests classes, e.g. *Carpino-Fagetea sylvaticae* (CA.FAG) and *Alno glutinosae-Populetea albae* (HA.POP). These adaptive trends were similar to those described globally both at the species-level (Díaz *et al*., 2016) and at the community-level (Bruelheide *et al.*, 2018), confirming the convergence of evolutionary history of individual species with community assembly. Whereas globally the size axis was the most relevant in explaining the total variance of species traits, we observed that the PC1-economics captured more of the total variance of our dataset explained by the two axes (42%). This result may be due to the presence of both woody and herbaceous species in the dataset analyzed by Díaz *et al.* (2016), and of woody and herbaceous communities in the dataset analyzed by Bruelheide *et al.* (2018), while we only considered woody species, which are known to be mainly differentiated by resource economics (e.g. Chave *et al.*, 2009; De La Riva *et al.*, 2016; Rueda *et al*., 2018).

Examination of the mean trait values for each of the 21 considered woody vegetation classes revealed that these were ordinated within the multidimensional space of the PCA according to four main physiognomic groups, as identified by the cluster analysis (Fig. 1b). Classes of evergreen heaths and scrubs, as well as evergreen forests, converged toward lower values of the size axis, thus exhibiting conservative leaf traits. These groups include classes dominated by needle-leaved and sclerophyllous species, typical of boreal/alpine zones, e.g. *Loiseleurio procumbentis-Vaccinietea* (AC.LOI), *Rhododendro hirsuti-Ericetea carneae* (CQ.RHO), *Vaccinio-Piceetea* (BA.PIC) and *Roso pendulinae-Pinetea mugo* (CP.MUG), or mediterranean climate, e.g. *Cisto-Lavanduletea stoechadis*

(FC.LAV), *Ononido-Rosmarinetea* (FB.ROS), *Junipero-Pinetea sylvestris* (CN.SAB) and *Quercetea ilicis* (FA.QUI), and therefore include species adapted to endure adverse environmental conditions such as drought, extreme temperatures (high and/or low) and prolonged snow cover (Caccianiga *et al*., 2006; Ciccarelli, 2014; De la Riva *et al*., 2016; Fyllas *et al*., 2020; Zanzottera *et al*., 2020). Indeed, both evergreen heath and forest classes showed a marked tendency toward the stress-tolerant (S) strategy (Fig. 2), confirming that in harsh habitats the environmental filter (i.e. abiotic stress) is a key determinant of the community functional composition, leading species to share similar traits as a common adaptation to physical constraints (Grime, 2006; De Bello *et al*., 2009). These results suggest a convergence toward a similar functional response of dominant diagnostic species of *syntaxa* that phytosociology considers to be completely different, being typical of distinct habitats.

Deciduous forest classes showed an overall prominent competitive strategy, with high mean values of C-selection (Fig. 2). These communities are typical of temperate climates, stable and productive environments with abundant resources, and occur under much less severe ecological constraints (Poorter *et al*., 2010; Kunstler *et al*., 2016; Costa-Saura *et al*., 2019). These habitat characteristics make environmental filters weaker, allowing more intra- and inter-specific interactions, and hence competition becomes the main driver shaping the functional character of the community (Navas and Violle, 2009; Grime and Pierce, 2012). Despite deciduous forest classes being very similar in their functional properties, it was possible to identify some differences. Some communities were dominated by species that denoted a lesser resource allocation to stem and seeds (i.e. low CANH and SM) and leaves with fast economics traits (i.e. high SLA and LNC) (Fig. 1b), which implies rapid growth and nutrient acquisition, typical of classes of wet environments (e.g. *Alnetea glutinosae* (IA.ALN)) or that could occur as pioneers during secondary succession in temperate forests (e.g. *Robinietea* (CF.ROB)) (Costa-Saura *et al*., 2019). Whereas, species of other classes showed a major investment in larger seeds, plant height and thicker leaves with slow resource economics characteristics (Fig. 1b), typical of classes corresponding to later successional stages (e.g. *Carpino-Fagetea sylvaticae* (CA.FAG), *Quercetea pubescentis* (CB.PUB) and *Quercetea robori-petraeae* (CC.QUE)) (Poorter *et al*., 2010; Adler *et al*., 2014).

The set of diagnostic species of *Robinietea* (CF.ROB), an alien-dominated class nonetheless recognized as part of European natural vegetation (Mucina *et al*., 2016; Viciani *et al*., 2020), exhibited the highest degree of competition among all the considered classes. In fact, all neophyte species were highly C-selected with a slight tendency toward the ruderal strategy (Fig. 2); these species showed effective resource acquisition traits, having large leaves with high nutrient concentrations (i.e. high SLA, LA and LNC), and rapid and extensive growth (i.e. high CANH) (Fig. 1b). Alien woody species tend to occupy disturbed environments, often as part of secondary succession (Richardson *et al*., 2000; Pyšek *et al*., 2017), and they are also efficient competitors that thrive in high-resource environments (Dalle Fratte *et al*., 2019). This confirms that the establishment of neophyte species is associated with ruderal and competitive strategies, whereas these species are not successful in heavily stressed habitats (Barni *et al*., 2012; Guo *et al*., 2018). Actually, Guo et al. (2018) observed that the naturalization incidence and extent of alien trees at the global scale were not correlated with either C-, S-, or R- scores, due to the low number of tree species in in the global pool of naturalized plants. Instead trees represented almost half of neophyte species (8 out of 15) in our dataset, two of them even showed the highest C- scores (*Trachycarpus fortunei* and *Ailanthus altissima*, Appendix S1), suggesting that the competitive strategy-naturalization success link is noticeable even in trees when only woody species are considered. Therefore, if an increment in disturbance and/or in resource availability should occur in classes typical of harsh-climate environments as a consequence of global change, favorable niches could become accessible to alien woody species that might significantly affect these communities (Davis *et al*., 2000; Funk, 2013; Dalle Fratte *et al*., 2019). For instance, montane regions are known to be particularly threatened by climate warming that facilitates alien species invasion, especially into already endangered habitats (Thuiller *et al*., 2008; Kleinbauer *et al*., 2010).

Our results demonstrated that the sets of woody species that are diagnostic for phytosociological classes mainly vary between S- and C-selection, as previously observed at the single species level at both European and global scales (e.g. Pierce *et al*., 2013, 2017). Indeed, all the sets of diagnostic species exhibited low mean values of R- selection, which indicates that they are not successful in habitats with a relatively high intensity of disturbance (Pierce *et al*., 2013). Indeed, Brzeziecki and Kienast (1994) classified some tree species as ruderal (9 out of 36 studied), considering them as pioneers that colonize gaps in the forest canopy (with a short lifespan, a high potential growth rate and a long-distance seeds dispersion), but Pierce et al. (2017) argue that pioneer trees are classified as C-selected because disturbance is not a regular occurrence (in the same way as annual mowing or grazing) and plants can attain large size without being killed. Thus ‘pioneer’ is not necessarily synonymous with ‘ruderal’. Moreover, in the global spectrum of plant form and function (Díaz *et al*., 2016) only herbaceous species are found at the most acquisitive end of the leaf economics axis, which corresponds with the ruderal strategy. Nonetheless, woody diagnostic species of deciduous shrub formations (*Salicetea purpureae* (HB.PUR) and *Betulo carpaticae-Alnetea viridis* (CR.VIR) displayed traits (i.e. small size and acquisitive resource economics) (Fig. 1b and Appendix S1) which reflected their greater adaptation to survive continuous destructive events typical of their habitat, due to high-energy water and snow flows, such as avalanches, snow creep, floods and waterlogging (Schnitzler *et al*., 1997; Leuschner and Ellenberg, 2017). Despite the fact that these two classes showed a tendency towards R-selection because of the disturbance they are exposed to, our results emphasize that woody vegetation classes display only low values of R- selection, which indicates that they are not successful in intensely disturbed habitats (Fig. 2 and Appendix 7), almost certainly because being a large perennial requires time and stability to accumulate biomass (see Pierce *et al.*, 2017).

We generally found a close relationship between the functional properties of woody diagnostic species, as defined by functional traits and CSR ecological strategies, and the physiognomy and the ecology of the classes they represent. This result represents progress toward the integration of phytosociology and functional ecology, which would also be valuable for the monitoring of certain Habitats (*sensu* Directive 92/43/CEE) that are often associated with particular *syntaxa* (Chytrý *et al.*, 2020). Indeed, a functional approach to the analysis of diagnostic species of such communities could improve structural and functional knowledge, as is required by the Habitat Directive (DG Environment, 2017).

5. Conclusion

In this study we found a general but clear correspondence between functional characteristics of woody diagnostic species of phytosociological classes of European woody vegetation and the physiognomy and ecology of the communities they represent. This confirms our hypothesis that functional traits and life strategies reliably indicate plant-environment relations in a manner that agrees with the phytosociological classification method. Such correspondence was particularly evident among classes of extreme environments (boreal/alpine and mediterranean) that showed a similar convergence toward species exhibiting conservative characteristics and the stress-tolerant strategy, indicating that a specific set of trait combinations is required to allow plant survival under harsh ecological conditions. More favourable and productive environments, rather, selected for species with a tendency toward the competitor strategy. Accordingly, the classes we examined exhibited an overall affinity for either the stress-tolerant or the competitor strategy, highlighting that abiotic stress and biotic competition are the main environmental drivers that affect them. No classes showed a marked ruderal strategy, supporting the idea that strong acquisitive characteristics are distinctive of other, non-woody, life forms that do not rely on achieving large size over many growth seasons. Neophyte woody species exhibited the same competitive strategy as that of native woody species, which implies that resource-limited or mildly disturbed environments that are currently occupied by native species could be the most exposed to alien species invasion, should a warming climate or nitrogen loads increase local productivity. We concluded that woody diagnostic species could be used to reliably indicate both the structure and the functional properties of woody vegetation classes coherently with their ecological characteristics.

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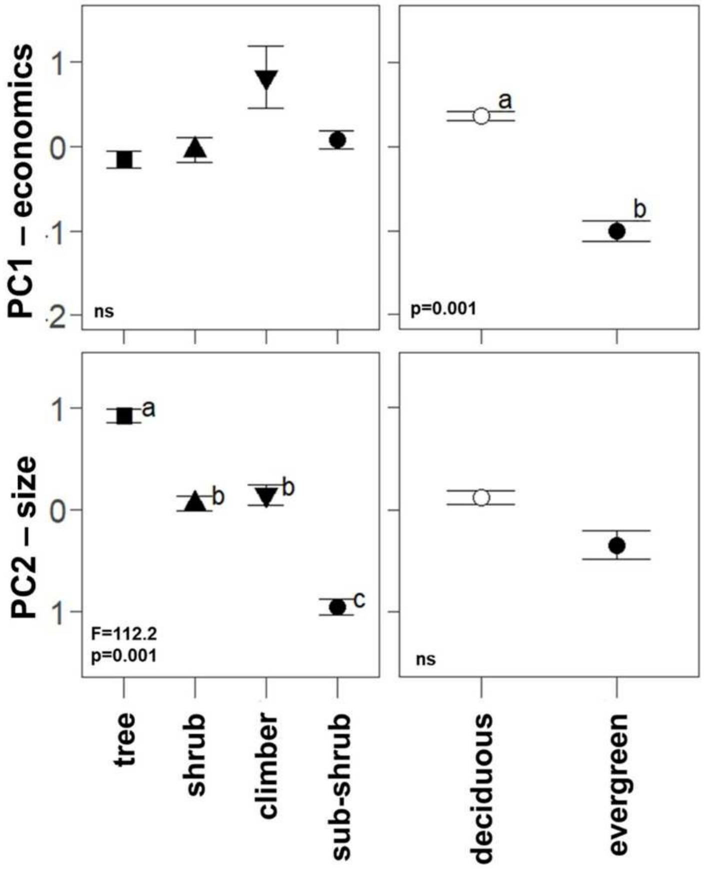
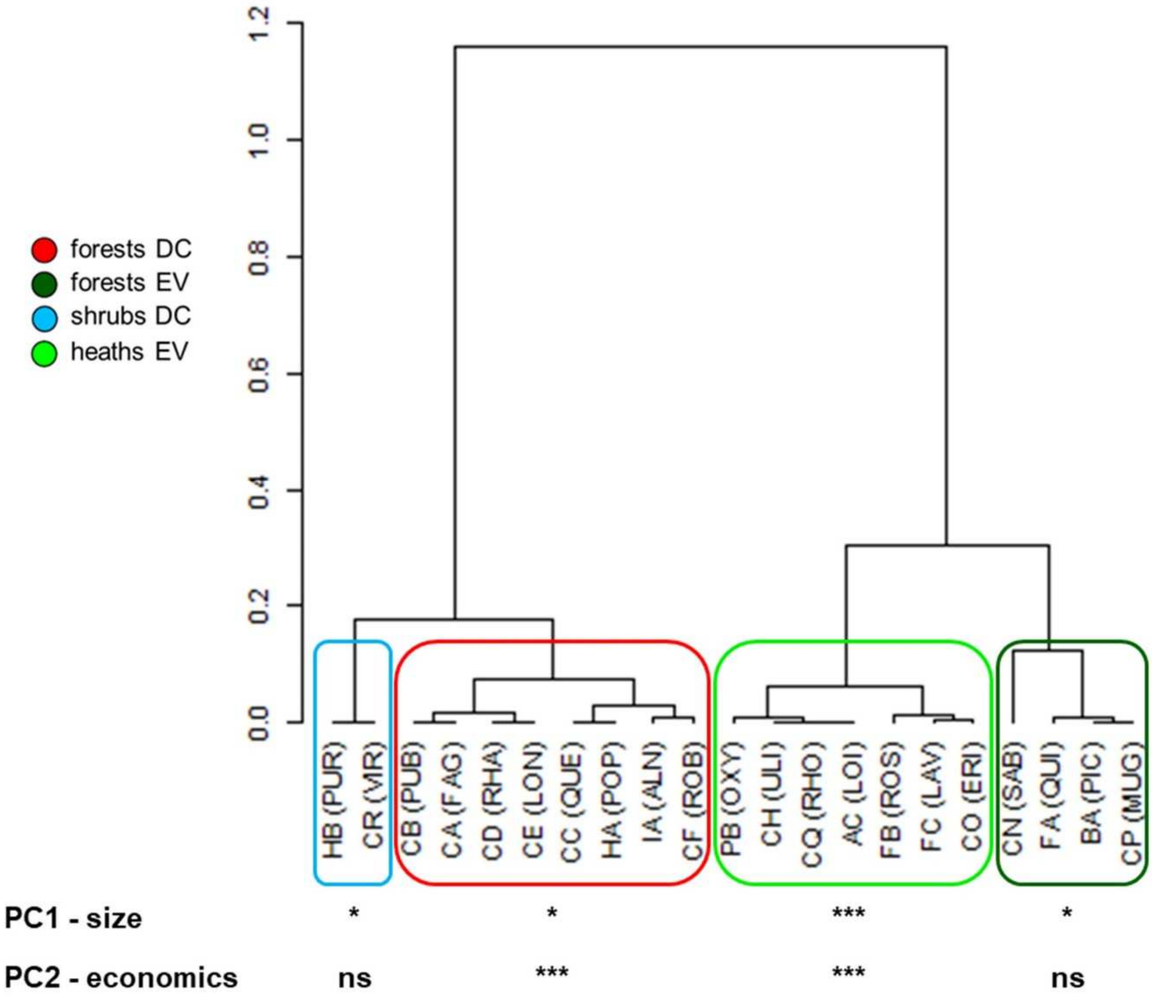
7. Supplementary material

Appendix S1: List of the 221 woody diagnostic species indicating their growth form, leaf life span, neophyte vs. native species, functional traits and Grime's CSR (Competitive, Stress-tolerant, Ruderal) plant strategies, and their distribution among the 21 classes of European woody vegetation considered for the analysis. Species names and classes refers to the EcoVeg checklist (Mucina et al., 2016) (full name of classes is reported in Table 1). Coordinate of sampling sites and biogeographic regions are also reported.

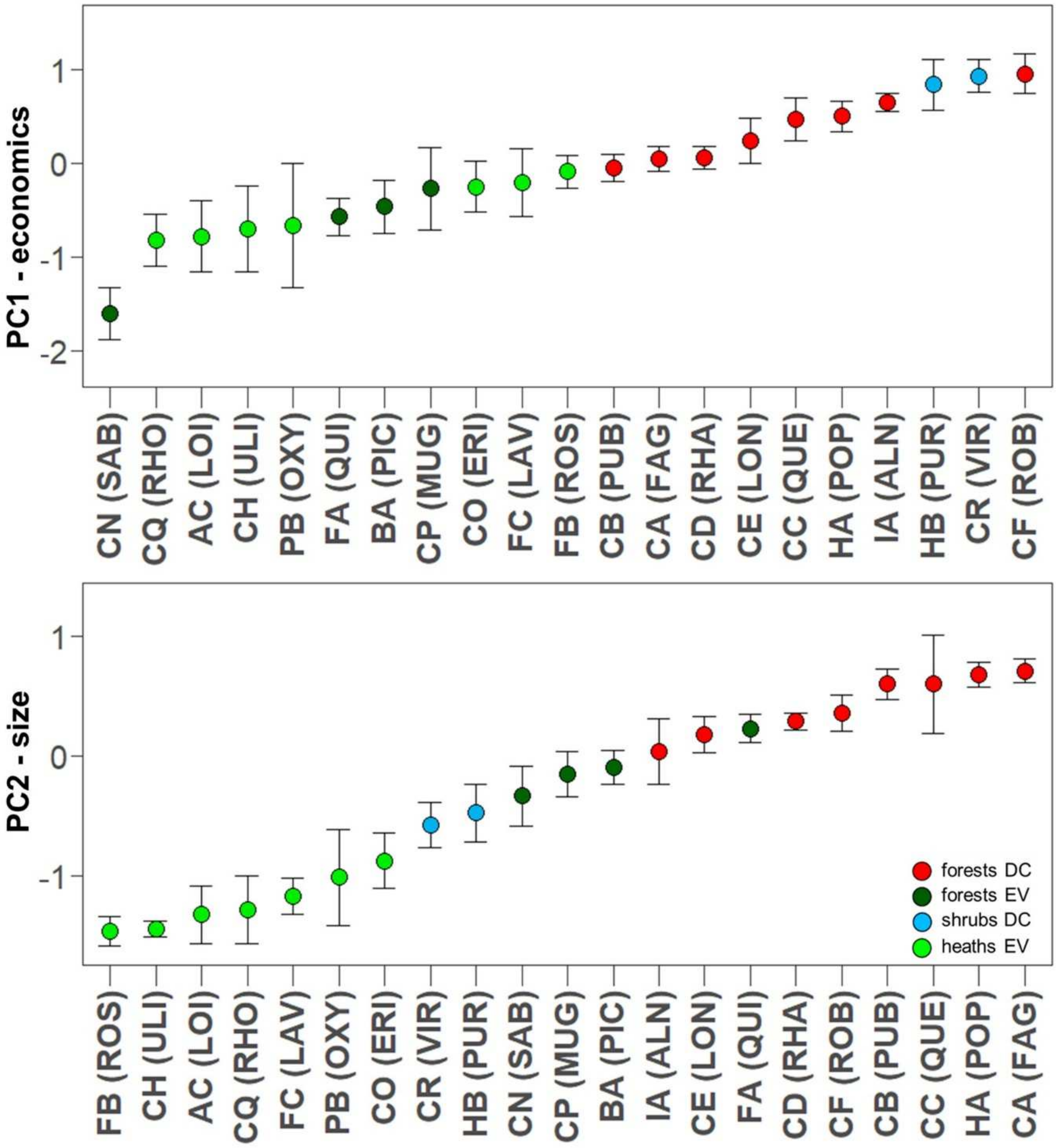
Appendix S2: Mean values (± se) of the scores of growth forms (left panels) and leaf life span (right panels) along the two axes of the principal component analysis (PC1 and PC2). Results of the phylogenetic ANOVA are also reported in each subplot; small letters indicate post-hoc comparisons.



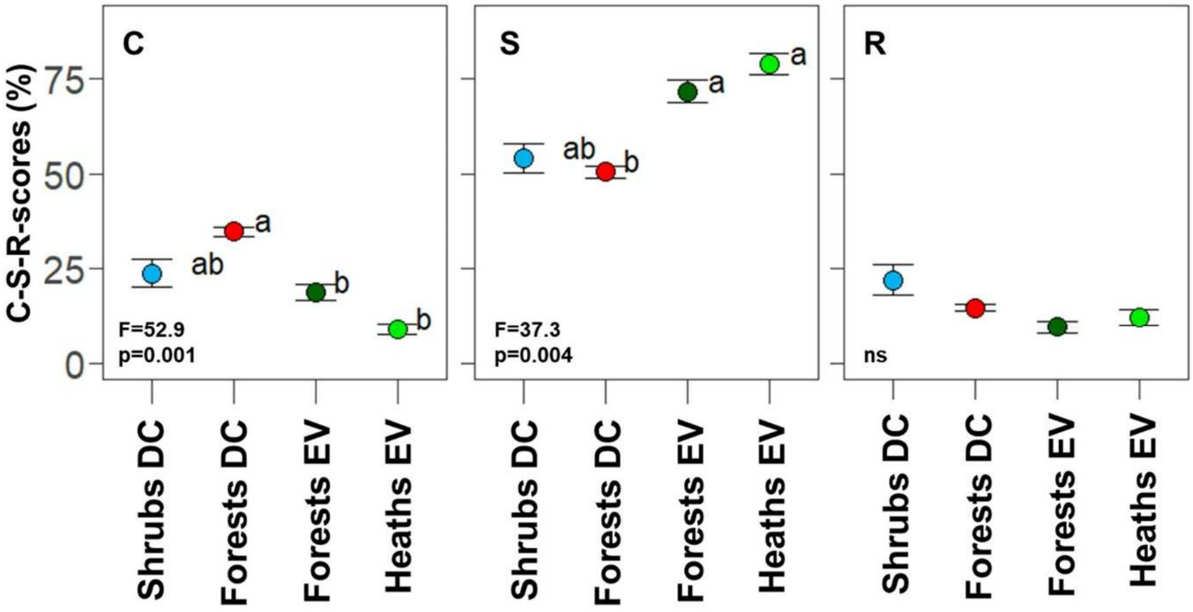
Appendix S3: Dendrogram resulting from the Hierachical Clustering on the mean values of Principal Components (PC1 and PC2) of diagnostic species of each class of European woody vegetation. Both axes contributed significantly ($p < 0.001$) to the clustering at $k = 4$, respectively Eta^2 = 0.92 and 0.72. The significance (p-value) of the contribution to each cluster by PC1 and PC2 is also reported in the figure. Classes (full names are reported in Table 1) are grouped into evergreen heaths and scrubs (light green), deciduous shrubs (light blue), evergreen forests (dark green) and deciduous forests (red). Legend: DC = deciduous, EV = evergreen, ns = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.



Appendix S4: Mean values (± se) of the scores of classes (full names are reported in Table 1) along the two axes of the principal component analysis (PC1 and PC2), grouped into evergreen heaths and scrubs (light green), deciduous shrubs (light blue), evergreen forests (dark green) and deciduous forests (red).



Appendix S5: Mean values (± se) of classes along C-, S- and R-scores. Results of the phylogenetic ANOVA are also reported in each subplot; small letters indicate *post-hoc* comparisons.



Appendix S6: Mean values (± se) of the scores of neophyte species and the native species considered along the two axes of the principal component analysis (PC1 and PC2) and C-, and S- scores. For each subplot, differences were always significantly different at $p < 0.001$ according to the phylogenetic ANOVA; R- scores did not show significant differences and are not shown.

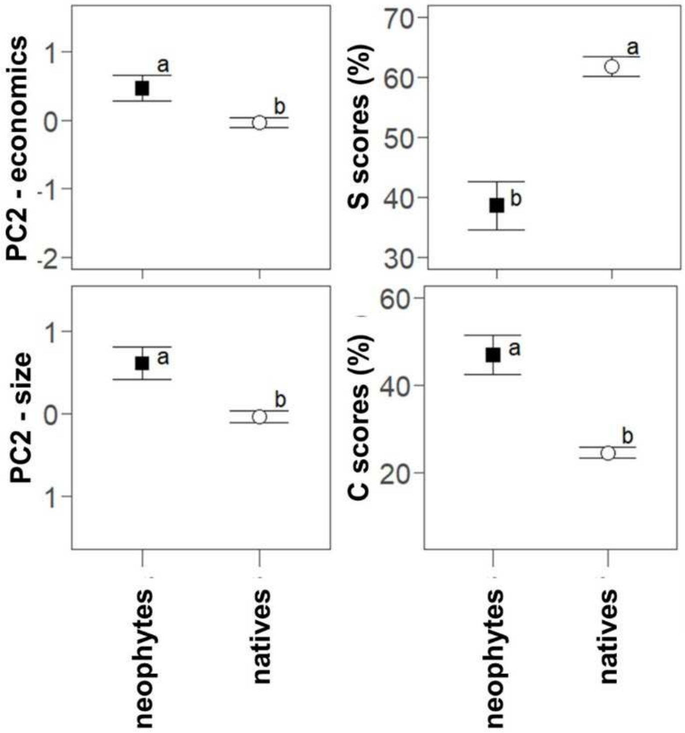


Table S1: the 21 vegetation classes considered, abbreviated according to the EcoVeg checklist (Mucina et al. 2016), with the diagnostic 221 species, their growth form and leaf life span and whether they are considered neophytes.

SPECIES	AC (LOI)	BA (PIC)	CA (FAG)	CB (PUB)	CC (QUE)	CD (RHA)	CE (LON)	CF (ROB)	CH (ULI)	CN (SAB)	CO (ERI)	CP (MUG)	CQ (RHO)	CR (VIR)	FA (QUI)	FB (ROS)	FC (LAV)	HA (POP)	HB (PUR)	IA (ALN)	PB (OXY)	NEOPHYTES	GROWTH FORM	LEAF LIFE SPAN
Abies alba	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Picea abies	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Larix decidua	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Pinus pinaster	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus nigra nigra	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus nigra laricio	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus sylvestris	.	x	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus mugo uncinata	.	x	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus mugo mugo	x	x	.	.	.	.	.	.	.	.	x	x	x	.	.	.	.	.	.	.	.	.	**shrub**	**evergreen**
Pinus mugo rotundata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	**shrub**	**evergreen**
Pinus halepensis halepensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus pinea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**tree**	**evergreen**
Pinus cembra	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Cupressus sempervirens	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**tree**	**evergreen**
Juniperus communis communis	.	x	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**evergreen**
Juniperus communis nana	x	.	.	.	.	.	.	.	.	x	.	x	x	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Juniperus oxycedrus oxycedrus	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**evergreen**
Taxus baccata	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**evergreen**
Salix pentandra	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Salix alba	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	**tree**	**deciduous**
Salix babylonica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	yes	**tree**	**deciduous**
Salix triandra triandra	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	**shrub**	**deciduous**
Salix myrsinifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	x	.	.	.	**sub-shrub**	**deciduous**
Salix glabra	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Salix appendiculata	.	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Salix cinerea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	**shrub**	**deciduous**
Salix capreae	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Salix rosmarinifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	**sub-shrub**	**deciduous**
Salix foetida	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Salix waldsteiniana	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Salix hastata	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Salix helvetica	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Salix eleagnos eleagnos	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	**tree**	**deciduous**
Salix purpurea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	**shrub**	**deciduous**
Salix caesia	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Populus alba	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**tree**	**deciduous**
Populus tremula	.	.	x	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Populus nigra	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**tree**	**deciduous**
Juglans regia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**tree**	**deciduous**
Betula pendula	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Betula pubescens sl	.	x	x	.	.	.	x	.	.	.	.	.	.	x	.	.	.	.	.	x	x	.	**tree**	**deciduous**
Alnus alnobetula alnobetula	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Alnus glutinosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	**tree**	**deciduous**
Alnus incana	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**tree**	**deciduous**
Alnus cordata	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Carpinus betulus	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Ostrya carpinifolia	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Corylus avellana	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**

Fagus sylvatica	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Castanea sativa	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Quercus ilex	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	tree	evergreen
Quercus suber	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	tree	deciduous
Quercus cerris	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Quercus petraea	.	.	x	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Quercus robur robur	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Quercus virgiliana	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Quercus pubescens	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Ulmus glabra	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Ulmus minor	.	.	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Ulmus laevis	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Celtis australis	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Ficus carica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	tree	deciduous
Osyris alba	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	sub-shrub	deciduous
Clematis flammula	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	climber	deciduous
Clematis vitalba	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	climber	deciduous
Clematis alpina	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	climber	deciduous
Berberis vulgaris	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Laurus nobilis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	tree	evergreen
Spiraea japonica	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	shrub	deciduous
Sorbaria sorbifolia	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	shrub	deciduous
Kerria japonica	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	shrub	deciduous
Rubus idaeus	.	.	.	.	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rubus ulmifolius	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rubus praecox	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rubus hirtus	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rubus caesius	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	sub-shrub	deciduous
Rosa rubiginosa	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rosa agrestis	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rosa pendulina	.	x	.	.	.	x	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rosa dumalis	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rosa canina	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rosa arvensis	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Pyrus communis communis	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Malus sylvestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Malus dasyphylla	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Sorbus domestica	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Sorbus aucuparia sl	.	x	.	.	.	x	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Sorbus torminalis	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Sorbus chamaemespilus	.	x	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Sorbus aria	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Amelanchier ovalis ovalis	.	.	.	x	.	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Cotoneaster nebrodensis	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Pyracantha coccinea	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Crataegus germanica	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	evergreen
Crataegus laevigata	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	shrub	deciduous
Crataegus monogyna	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Prunus cerasifera	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Prunus spinosa	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Prunus avium	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Prunus cerasus	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Prunus mahaleb	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Prunus padus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Prunus serotina	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	tree	deciduous
Prunus laurocerasus	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Cercis siliquastrum	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Laburnum anagyroides	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Laburnum alpinum	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous

Cytisus spinosus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	shrub	deciduous
Cytisus nigricans nigricans	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Cytisophyllum sessilifolium	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Cytisus emeriflorus	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Cytisus scoparius	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	evergreen
Cytisus purpureus	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Cytisus hirsutus hirsutus	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Genista tinctoria sl	.	.	.	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Genista pilosa	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Genista germanica	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Genista radiata	.	.	.	.	.	.	.	.	.	x	x	.	x	.	.	.	.	.	.	.	.	.	sub-shrub	evergreen
Robinia pseudoacacia	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	tree	deciduous
Colutea arborescens	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Dorycnium hirsutum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	evergreen
Dorycnium pentaphyllum pentaphyllum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	evergreen
Hippocrepis emerus emerus	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Euphorbia dendroides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	sub-shrub	evergreen
Euphorbia characias	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	sub-shrub	evergreen
Ailanthus altissima	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	tree	deciduous
Polygala chamaebuxus	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Coriaria myrtifolia	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Cotinus coggygria	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Pistacia terebinthus	.	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	shrub	deciduous
Pistacia lentiscus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	shrub	deciduous
Acer platanoides	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Acer campestre	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	tree	deciduous
Acer pseudoplatanus	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Acer opalus opalus	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Acer negundo	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	tree	deciduous
Acer saccharinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	yes	tree	deciduous
Aesculus hippocastanum	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Ilex aquifolium	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	evergreen
Euonymus europaeus	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	deciduous
Buxus sempervirens	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	shrub	evergreen
Rhamnus alaternus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	shrub	deciduous
Rhamnus saxatilis saxatilis	.	.	.	.	.	x	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Rhamnus carthartica	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	shrub	deciduous
Frangula alnus	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	shrub	deciduous
Parthenocissus quinquefolia	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	climber	deciduous
Tilia platyphyllos	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Tilia cordata	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Tilia tomentosa	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	tree	deciduous
Daphne mezereum	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Daphne gnidium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	shrub	deciduous
Daphne laureola	.	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Daphne cneorum	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Daphne striata	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	sub-shrub	deciduous
Hippophaë rhamnoides fluviatilis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	shrub	deciduous
Elaeagnus pungens	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	shrub	evergreen
Cistus albidus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	sub-shrub	deciduous
Cistus creticus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	sub-shrub	deciduous
Cistus monspeliensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	sub-shrub	deciduous
Cistus salviifolius	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	sub-shrub	deciduous
Helianthemum apenninum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	evergreen
Helianthemum italicum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	evergreen
Fumana ericoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	deciduous
Fumana thymifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	deciduous
Fumana laevipes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	sub-shrub	deciduous
Myricaria germanica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	shrub	deciduous

Myrtus communis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**evergreen**
Punica granatum	.	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**deciduous**
Cornus sanguinea	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Cornus mas	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Hedera helix	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**climber**	**deciduous**
Erica arborea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**evergreen**
Erica carnea	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Erica scoparia	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**evergreen**
Calluna vulgaris	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	x	.	.	.	x	.	**sub-shrub**	**evergreen**
Rhododendron ferrugineum	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Rhododendron hirsutum	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Rhodothamnus chamaecistus	.	.	.	.	.	.	.	.	.	.	x	.	x	.	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Kalmia procumbens	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Arbutus unedo	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**evergreen**
Arctostaphylos uva-ursi	.	x	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Arctostaphylos alpina	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Andromeda polifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	**sub-shrub**	**deciduous**
Vaccinium vitis-idaea	x	x	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Vaccinium uliginosum	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	**sub-shrub**	**deciduous**
Vaccinium gaultherioides	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Vaccinium myrtillus	.	x	.	.	x	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Empetrum nigrum	x	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Empetrum hermaphroditum	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Fraxinus ornus	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Fraxinus excelsior	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**tree**	**deciduous**
Fraxinus angustifolia oxycarpa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**tree**	**deciduous**
Syringa vulgaris	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Ligustrum vulgare	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Ligustrum lucidum	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	**tree**	**evergreen**
Olea europaea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**tree**	**evergreen**
Phillyrea angustifolia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**evergreen**
Phillyrea latifolia	.	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**evergreen**
Teucrium capitatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	**sub-shrub**	**deciduous**
Thymus vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Rosmarinus officinalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Lavandula stoechas	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.	.	**sub-shrub**	**evergreen**
Buddleja davidii	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	**shrub**	**deciduous**
Sambucus nigra	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**shrub**	**deciduous**
Sambucus racemosa	.	.	.	.	.	.	.	x	.	.	.	.	.	x	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Viburnum lantana	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**shrub**	**deciduous**
Viburnum tinus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**shrub**	**deciduous**
Viburnum opulus	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	**shrub**	**deciduous**
Lonicera caerulea	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Lonicera alpigena	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Lonicera nigra	.	x	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Lonicera xylosteum	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**shrub**	**deciduous**
Lonicera japonica	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	**climber**	**deciduous**
Lonicera implexa	.	.	.	.	.	x	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**climber**	**evergreen**
Lonicera caprifolium	.	.	x	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**climber**	**deciduous**
Helichrysum italicum italicum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Helichrysum stoechas stoechas	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Asparagus acutifolius	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Ruscus aculeatus	.	.	.	x	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**sub-shrub**	**evergreen**
Smilax aspera	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	**climber**	**evergreen**
Trachycarpus fortunei	.	.	x	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	yes	**tree**	**evergreen**

Conclusions

This Ph.D. project allowed an overall better insight into plant ecology and functioning. The main focus was on the functional description of a wide range of plant communities from different habitats through the application of plant functional traits and Grime's CSR plant adaptive strategies, with the final goal to identify trends and responses to environmental factors.

The study started from the species-level, analysing trait interactions, trait variation along ecological gradients and species adaption and success to new environments; then, it moved to the community-level comparing traits and adaptive strategies of multiple plant communities from the same site, the same environment and ecologically distant.

We found that other leaf traits could be considered alongside the more frequently used core traits to describe relevant functions within the leaf economics spectrum, particularly those related to leaf sulfur content (i.e. LSC and C:S), given the importance of this nutrient for plant functioning (e.g. in relation to photosynthetic capacity, respiration rates and secondary metabolites); such traits could also provide better understanding of processes and patterns of ecosystems in response to environmental changes as its biochemical cycle is largely impacted by human activities. Besides nutrient availability, global change is affecting other ecological characteristics, such as soil properties, temperature and climate in general; we observed the different responses of a wide range of species to changes in such drivers and suggested that future global change could promote the establishment and development in Southern Europe of non-native species belonging to functional groups not currently present, especially invasive ones. Indeed, we also found that invasive alien species possess combinations of traits associated with rapid resource acquisition and growth, which makes them true competitors that can outcompete native species in their same habitat. These results showed the importance of functional traits and adaptive strategies to explain plant persistence in a given environment in relation with ecological factors and predict their response to possible changes.

Furthermore, from the functional analysis (through CWMs of functional traits and CSR strategies) of multiple communities along a topographic sequence, we could observe the high vegetation variability as a response to the variation of ecological factors, which was also noticeable when considering floristically and ecologically defined communities (i.e. Habitats of EU Community interest) along a successional gradient at the regional scale. These results provided the functional signature of the vegetation as a whole which is not so evident among other studies, especially when considering CSR plant strategies; moreover, we confirmed that trait trade-offs between communities evident at the global scale are mainly affected by local environment adaptions.

Finally, one particularly relevant finding of this project was the identification of a relation between two plant species and communities description and classification methods that have been widely applied over the last century: phytosociology and functional ecology; this was evident from the analysis of functional traits and life strategies of diagnostic species of woody vegetation classes that reliably indicated plant-environment relations according to their physiognomy and ecology. This result could indicate a first step toward an explicit connection between phytosociology and functional ecology, which is a gap that still needs to be filled to achieve a comprehensive understanding of species-environment relations. According to our findings the functional approach could be incorporated in current vegetation classification systems that are only based on taxonomy. Moreover, diagnostic species proved to efficiently represent both function and structure of plant communities and, thus, could be used for their monitoring as required by EU Directive (92/43/CEE).

On the basis of the results of this project, further research on the functionality of plant communities might provide a deeper understanding of plant assembly rules along ecological gradient, especially in alpine habitats. Similarly, it could be valuable to determine and compare the functional fingerprint of such communities through functional diversity indices. These analyses could finally lead to identify some of the vegetation functional characteristics that particularly affect ecosystem properties and, therefore, the quantity and the quality of ecosystem services toward their proper assessment and conservation.

Appendix 1

Dataset and traits measurement

The datasets used for this PhD project were collected from the database of the regional Biodiversity Observatory of Lombardy (www.biodiversita.lombardia.it), which collects 6,137 phytosociological *relevès*, 4,730 of which are referred to 54 Habitats of EU Community interests (Directive 92/43/CEE) present in Lombardy, and that includes over 2,236 plant *taxa* (2,006 vascular plants). For almost all of these taxa (n = 1,523), several functional traits were measured and are available in the global functional trait database TRY (Kattge et al. 2020), other than in the LIFTH (Dalle Fratte et al. 2020) and FIFTH (Cerabolini et al. 2010) datasets.

Traits were measured following a standardized methodological protocol (Perez-Harguindeguy et al. 2016); specifically, for each species 5 to 15 fully expanded leaves were sampled randomly within the same population from the outer canopy of different individual adult plants growing under optimal conditions. Leaves were accurately washed and stored wrapped in wet paper towels and tin foil at 4°C for 24 hours to allow maximum expansion and hydration. Then, leaf fresh (LFW) weight was taken, and leaf area (LA) was measured through scanned images analysed by the software Leaf Area Measurement (LAM v1.3). Leaf dry weight (LDW) was taken after drying the leaves at 70°C overnight. Specific leaf area (SLA) and leaf dry matter content were then calculated as LA to LDW ratio and LFW to LDW ratio, respectively.
Afterwards, leaves were pounded using pestles with liquid nitrogen and analyzed through gas chromatography in the CHNS-analyzer "Flash EA 1112 series Thermo Fisher Scientific" doing three replicates for each species; leaf carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) content (LCC, LHC, LNC, LSC respectively) were then obtained (Fadeeva et al. 2008).

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Appendix 2

Study area

All the species and the phytosociological *relevès* considered in this project are from Lombardy, which is an administrative region in the North of Italy that covers an area of 23,870 Km^2 ranging between latitudes 44°40'– 46°37' N and longitudes 8°29' – 11°25' E. This large territorial extension allows to include within its borders areas with complex morphology and orography, from the Alps (reaching altitudes of approximately 4.000m a.s.l.) to the Po alluvial plain (10m a.s.l.). Geological substrates consist of a wide range of litho-types, both silicate or carbonate rocks, alluvial or morainic deposits. In such a variegated territory and climate conditions, almost all European vegetation belts are represented, from alpine tundra with periglacial species to deciduous forests and wetlands, and even Mediterranean holly oak woods are present on the shores of the Lake of Garda.

In Lombardy region are present 58 Habitats of EU Community Interest (Directive 92/43/CEE), 16 of which are also of primary importance for conservation as they require particularly accurate monitoring and management. In a recent work by Dalle Fratte et al. (2019) the distribution of EU Habitats has been mapped at the regional scale, both within and outside Natura 2000 network sites which cover 21.9% of the regional territory (n = 196 Sites of Community Importance and n = 67 Specially Protected Areas).

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Dalle Fratte, M., Brusa, G. and Cerabolini, B.E.L. (2019). A low-cost and repeatable procedure for modelling the regional distribution of Natura 2000 terrestrial habitats. Journal of Maps, 15(2): 79-88.

PhD side-activities

Summer schools

- Habitat monitoring (6130, 7220*, 9130) – project Oltrepò Bioviderso
  2-6 July 2018 – Romagnese, PV.
  Lecturers: S. Assini (University of Pavia), M. Barcella (University of Pavia), M. Mariotti (University of Genoa), S. Poponessi (University of Perugia), R. Venanzoni (University of Perugia).

- International Trait School – Functional Traits of Organisms
  19-24 March 2019 - Centre d'Etude de la Foret, Ile de Porquerolles, France.
  Lecturers: F. De Bello (University of South Bohemia), S. Delzon (Institut National de la Recherche Agronomique), E. Garnier (Centre National de la Recherche Scientifique - Centre d'Ecologie Fonctionnelle et Evolutive), E. Kazakou (Centre d'Ecologie Fonctionnelle et Evolutive), A. Munson (Laval University), J. Posada (Del Rosario University), B. Shipley (University of Sherbrooke).

Conferences and Workshops

- 2nd International Conference on Community Ecology
  4-6 June 2019, Bologna.

- Conference of Young Botanists – CYBO
  6-7 February 2020, Genoa.

- Italian Society of Vegetation Sciences (SISV) Online Workshop – From phytosociology to Habitat monitoring: character species, diagnostic species and typical species
  9 October 2020, online.

Posters

- Zanzottera, M., Brusa, G., Caccianiga, M., Dalle Fratte, M. and Cerabolini, B.E.L. (2018). The Lombardy database of relevés concerning Habitats of Community interest (Directive 92/43/EEC), a keystone to improve the knowledge about them at a regional scale. Acta of 52° Italian Society of Vegetation Sciences (SISV) Congress, Catania, 5-7 April 2018.

- Zanzottera, M., Caccianiga, M., Dalle Fratte, M. and Cerabolini B.E.L. (2019). Functional characterization of alpine habitats (Directive 92/43/EEC) by community weighted mean of leaf traits: a preliminary study. Acta of 53° Italian Society of Vegetation Sciences (SISV) Congress, Sassari, 30 May-1 June 2019.

Communications

- Zanzottera, M., Caccianiga, M., Dalle Fratte, M. and Cerabolini, B.E.L. (2019). Plant strategies and functional diversity along communities successional gradients in alpine environment. 2° International Conference on Community Ecology, Bologna, 4-6 June 2019.

- Zanzottera, M., Caccianiga, M., Dalle Fratte, M. and Cerabolini, B.E.L. (2020). Functional phytosociology of woody Italian vegetation. Conference of Young Botanists (CYBO), Genova, 6-7 February 2020.

- Zanzottera, M., Dalle Fratte, M., Caccianiga, M. and Cerabolini, B.E.L. (2020). Functional Phytosociology, is it possible? A test on diagnostic species of European woody vegetation in the seek of typical species. Italian Society of Vegetation Sciences (SISV) Online Workshop, 9 October 2020.

Conference papers

- Dalle Fratte, M., Pierce, S., Zanzottera, M. and Cerabolini, B.E.L. (2020). Plant traits and ecological strategies in the dark diversity of forest vegetation in the province of Varese (Lombardy). 115th Italian Botanical Society (SBI) Congress, 9-11 September 2020.

- Dalle Fratte, M., Acosta, A.T.R., Caccianiga, M., Carboni, M., Ricotta, C., Zanzottera, M. and Cerabolini, B.E.L. (2020). From abundance-based to functional-based diagnostic species. Italian Society of Vegetation Sciences (SISV) Online Workshop, 9 October 2020.

Non-IF Journals publications

- Brusa, G., Dalle Fratte, M., Zanzottera, M. and Cerabolini B.E.L. (2017). Come implementare la conoscenza floristico-vegetazionale in Lombardia? La banca dati degli habitat di interesse comunitario (Direttiva 92/43/CEE). Natura Bresciana, 41: 45-66.
- Dalle Fratte, M., Brusa, G., Zanzottera, M. and Cerabolini, B.E.L. (2018a). Valutazione degli habitat di interesse comunitario (Direttiva 92/43/CE) nei Siti Rete Natura 2000 della Lombardia: gli habitat sovrapposti. Università degli Studi dell'Insubria - Fondazione Lombardia per l'Ambiente, Osservatorio Regionale per la Biodiversità di Regione Lombardia.

- Dalle Fratte, M., Brusa, G., Zanzottera, M. and Cerabolini, B.E.L. (2018b). Protocollo di segnalazione delle modifiche cartografiche degli habitat di interesse comunitario (92/43/CEE) presenti all'interno di Rete Natura 2000 in Lombardia. Università degli Studi dell'Insubria - Fondazione Lombardia per l'Ambiente, Osservatorio Regionale per la Biodiversità di Regione Lombardia.

- Brusa, G., Dalle Fratte, M., Zanzottera., M. and Cerabolini, B.E.L. (2019a), Cambiamenti a lungo termine dell'uso del suolo nell'area di Malpensa e conseguenze sull'attuale presenza della brughiera. Pianura-Scienze e Storia dell'Ambiente Padano, 38: 72-85.

- Brusa, G., Dalle Fratte, M., Armiraglio, S., Ceriani, R.M., Zanzottera, M. and Cerabolini, B.E.L. (2019b). Flora e habitat di interesse comunitario (Direttiva 92/43/CEE) in Lombardia: sintesi della distribuzione e importanza di conservazione. Natura Bresciana, 42: 91-102.

- Dalle Fratte, M., Zanzottera, M. and Cerabolini, B.E.L. (2020). Valutazione degli habitat di interesse comunitario (Direttiva 92/43/CE) nei Siti Rete Natura 2000 della Lombardia: distribuzione ambientale e identificazione delle soglie dei parametri per la valutazione dello stato di conservazione degli Habitat 6230*, 6240* e 6520. Università degli Studi dell'Insubria - Fondazione Lombardia per l'Ambiente, Osservatorio Regionale per la Biodiversità di Regione Lombardia.

Bachelor thesis tutoring

- Daniele, L. (2019). Caratteri fogliari delle specie caratterizzanti le formazioni arboreo-arbustive della vegetazione italiana. Cerabolini, B.E.L (supervisor) and Zanzottera, M. (tutor). Defended on 11 December 2019 at University of Insubria, Varese.

- Mattiazzo, F. (2020). Comparazione dei caratteri fogliari di specie legnose autoctone e alloctone presenti in Lombardia. Cerabolini, B.E.L (supervisor) and Zanzottera, M. (tutor). Defended on 10 June 2020 at University of Insubria, Varese.

IF Journals Publications

- Dalle Fratte, M., Brusa, G., Zanzottera, M. and Cerabolini, B.E.L. (2019a). Plant traits variation along ecological gradients denoted by environmental indicators. Flora - Morphology, Distribution, Functional Ecology of Plants, 254: 113-121.

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- Ferrè, C., Caccianiga, M., Zanzottera, M. and Comolli, R. (2020). Soil–plant interactions in a pasture of the Italian Alps. Journal of Plant Interactions, 15(1): 39-49.

- Zanzottera, M., Caccianiga, M., Dalle Fratte, M., Pierce, S. and Cerabolini B.E.L. (2020a). Community level variation in plant functioning shapes habitat structure along succession gradients in an alpine environment. Community Ecology, 21: 55-65.

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