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Analysis of the functional traits of *Quercus cerris* L. seedlings in the Molise region (southern Italy)

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Abstract

This study deals with the analysis of seedling fitness in three *Quercus cerris* wood stands, namely Selva di Castiglione (SC), Bosco della Ficora (BF) and Bosco di San Leo (BSL), developed in different lithological and physiographic conditions and subjected to different forestry practices. A phytosociological study was carried out for analysing the coenological features of the forest stands and to classify these latter from a syntaxonomic point of view. The Pignatti-Ellenberg index (PEi) was calculated on the matrix composed of the phytosociological relevés in order to highlight possible ecological differences or gradients among stands. The phenotypic parameters considered were the stem and root length and the leaf area, whereas the plant functional traits (PFTs) were specific leaf area (SLA), leaf dry matter content (LDMC), leaf thickness (Lth) and chlorophyll content (CHL). The results showed that seedlings coming from different sampling sites exhibited similar values in all the phenotypic parameters. Instead, statistically significant differences were observed in the PFTs. The results suggested that the different adaptation strategies implemented by the seedlings are to be related to the physical environment of the sampling sites and to the different forest structures. The Selva di Castiglione forest stand (SC) exhibited better growth conditions for seedlings testified by higher values of SLA and CHL and lower values of LDMC and Lth. These were interpreted as greater investment in carbon production aimed at rapid development and renewal of the seedling rather than carbon storage aimed at ensuring leaf longevity.

Keywords

functional trait, high forest, oak, phenotypic response, phytosociology, southern Apennines

Introduction

Quercus cerris L. (Turkey oak, Fagaceae) acts as a dominant species in a large part of the oak forests of central and southern Italy (Blasi et al. 2004; Di Pietro et al. 2020b; Terzi et al. 2021). In the Molise region, the *Q. cerris* forests are widely distributed within the hilly, submontane and lower montane belt, ranging in altitude between 300 and 1200 m a.s.l. (Blasi and Paura 1995; Blasi et al. 2004, 2005). In particular, a wide *Q. cerris* belt separates thermophilous mixed oak woods with *Q. cerris*, *Q. pubescens* Willd. and *Q. frainetto* Ten. of the hilly belt from the beech woods of the montane belt. The *Q. cerris* belt is linked to Flysch substrates or the occurrence of large karst basins on limestone bordered by mild slopes covered by leached rich in clay soils. However, the abundance of *Q. cerris* woods in the Molise region is also due to the application of forestry management (e.g., different forest systems and silvicultural systems) that have advantaged this species over other tree species (e.g., *Q. petraea* (Matt.) Liebl. and *Q. frainetto*). The high-forest system of the Turkey oak woods, which is typically adopted in many parts of Italy and especially in the Molise region, finds its historical reason in the production of railway sleepers and, only marginally, of firewood (Di Martino 1996). Owing to the importance of Turkey oak for the regional economy, and paying attention to the ongoing climate change, we carried out an in-situ study aimed at identifying possible phenotypic response and plant functional traits (PFTs) variations from *Q. cerris* seedlings coming from wood stands characterised by different environmental conditions and forestry treatment. PFT variations have proved to be useful for establishing ecological and evolutionary trends for plant species and communities. They have often been used to interpret plant-environment relationships, to make previsions on possible consequences and to quantify a wide range of natural and/or human-driven processes in terms of species or community ecology (Pèrez-Harguindeguy et al. 2013; Adler et al. 2014; Pérez-Ramos et al. 2019). The measurements of the functionality patterns of seedlings of *Q. cerris* growing under different forest management, could provide useful information on the health state of the woods as well as address forest management policies in the next future. Surprisingly the available literature on such studies (i.e., observations in situ on *Q. cerris* seedlings) is relatively scarce in Italy. Most of the ecological studies performed on *Q. cerris* dealt with adult trees (e.g., Gratani and Foti 1998) or analysed, in greenhouses or under controlled conditions, the response of woody species seedlings to various types of induced stress (Otieno et al. 2005; Aref et al. 2013; Karavin et al. 2013; Chiatante et al. 2015; Abdala-Roberts et al. 2018; Torres-Ruis et al. 2019). Other studies have focused on the phenotypic plasticity of a species as a measure of its adaptability and fitness to environmental and climatic changes (Valladares et al. 2000, 2014; Pérez-Ramos et al. 2019). Field studies on the vitality of plants using PFTs, however, are more frequently applied to grassland communities (Bolzan et al. 2007; Catorci et al. 2013; Chelli et al. 2019; Baltieri et al. 2020) than to forest ones.

This study is part of an interdisciplinary and broader line of research that has been undertaken by the Laboratory of Systematic Botany and Floristics of the University of Molise in the last decade, which aims to shed light on the morphological, ecological, and bio-molecular features of *Quercus* species (Antonecchia et al. 2015; Fortini et al. 2015a, 2015b; Di Pietro et al. 2016; Conte et al. 2019; Di Pietro et al. 2020a, 2020c; 2021). Specifically, the present paper aims to evaluate the response of *Quercus cerris* seedlings to different ecological and forest management conditions, using PFTs and the analysis of morphological trait variations.

Materials and methods

Study area

The study was carried out in three *Quercus cerris* woods located in the submontane-lower montane belt of the Molise Region (Italy), namely Selva di Castiglione (SC), Bosco di San Leo (BSL), Bosco della Ficora (BF) (Fig. 1). According to Blasi et al. (2010) and Rivas-Martínez et al. (2011), the macro-bioclimate of the study area can be defined as temperate oceanic/sub-Mediterranean while based on the classifications of the Ecoregions of Italy (Blasi et al. 2018), the study area falls under code 1C3a1. The code identifies the territory according to the following characteristics: 1 = Temperate Division, C = Apennine Province, 3 = Southern Apennine Section, a = Campanian Apennine Subsection. The first wood stand (Selva di Castiglione–SC) is developed within the bottom of a plain on clayey substrates, while the other two stands (Bosco di San Leo–BSL and Bosco della Ficora–BF) are located within slopes with declivity on arenaceous-pelithic substrate. Physiographic information on the sites of collection is summarised in Table 1.

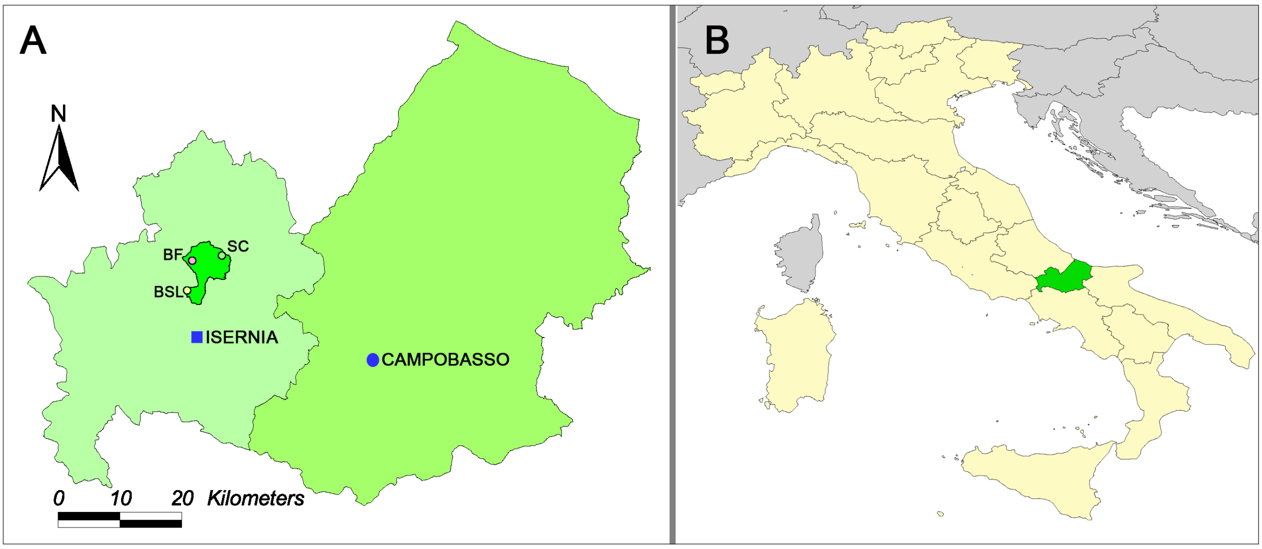

Figure 1. A) Location of the three sampled sites (Municipality of Carovilli) in the Province of Isernia in the Molise Region. BF = Bosco della Ficora, BSL = Bosco di San Leo, SC = Selva di Castiglione. B) Location of the Molise Region in Italy.

Table1. Characteristics of the sampled sites (Cantiani et al. 2010).

	Coordinate (DMS)	Stand development	Age	Altitude (m a.s.l.)	Aspect	Slope (%)	Substrate
Bosco di San Leo (BSL)	41°40'33"N 14°15'18"E	Mature high forest	135	810	NW	15	arenaceous-pelithic
Bosco della Ficora (BF)	41°42'55"N 14°18'55"E	Young high forest	55	920	N-NW	15	arenaceous-pelithic
Selva di Castiglione (SC)	41°44'12"N 14°18'50"E	Adult high forest	90	1000	-	0	varicoloured clay

Silvicultural description

The sampled stands have already been the subject of experimentation on the *Q. cerris* forests by the Council for Research and Analysis of Agricultural Economics (CREA). From a forestry point of view, the three stands have different histories and are currently passing through different silvicultural stages. The BSL stand is an old forest (135 years) and is a result of a natural regeneration stage starting from a senescent high forest subjected to thinning interventions. The average diameter of Turkey oak individuals ranges between 30 and 35 cm. The BF stand is a young high forest (average age 55 years), which having never been subjected to silvicultural interventions, is currently characterised by an excessive density of tree individuals. The SC stand is an adult forest (average age ranging between 60 and 100 years). In 2006 and 2007, the following silvicultural treatments were applied to the three forest stands (Cantiani et al. 2010): BSL, shelterwood cutting (seed-tree); SC, high thinning (grade: heavy) and BF, selective thinning.

Data and methods

To obtain coenological information on the three *Q. cerris* stands a field-sampling campaign was carried out during the 2019 summer period using the phytosociological approach (Braun-Blanquet 1964). Species and syntaxa nomenclature in the phytosociological table followed Bartolucci et al. (2018) and Biondi et al. (2014), respectively. The following Ellenberg indicator values (L - light availability; T - temperature) and edaphic factors (U - soil moisture; N - soil nutrient) as reported in Pignatti (2005) were also considered to infer possible ecological differences between the three *Q. cerris* stands investigated. For the indicator values that were missing for some species in Pignatti (2005), reference was made to Sürmen et al. (2014). Subsequently, Pignatti-Ellenberg index was applied to the matrix of phytosociological relevés by calculating the weighted averaging using species abundances to evaluate the average response of each forest community to the abiotic factors associated with Ellenberg indicator values, following what is reported in Gristina and Marcenò (2008) from the unpublished data of Pignatti and Bona (2007).

The in-field collection of *Quercus cerris* seedlings followed mainly the protocol reported in Gottardini et al. (2016) according to the following steps: 1) identification of a sampling stand, with a random collection of thirty 3 to 4-year-old seedlings, starting from the centre of the site and maintaining a distance of 6–7 m between each seedling collected, choosing seedlings with fully expanded leaves free from damage from herbivores or pathogens (Garnier et al. 2001); 2) eradication of the seedlings by taking an entire clod of soil with a spade up to 30 cm deep and subsequent removing of the soil from the root through water; 3) measurements of the soil pH (classes of pH soil according to National Soil Survey Center 1998) precisely where the individual seedlings were eradicated; 4) measurement of the bulk density of the soil through a metal cylinder (classes of bulk density soil according to National Soil Survey Center 1998); 5) application of a damp cotton cover to each seedling and store it in dark in the cooler and 6) measurements of the chlorophyll leaf content by the at LEAF CHL PLUS instrument (FT Green LLC, Wilmington, US), whose function is comparable to the SPAD 502 (Minolta) (Zhu et al. 2012; Novichonok et al. 2016). The measurements were repeated three times in the central part of the lamina of three leaves directly in the field.

The first stage of field sampling was carried out during three consecutive days (24, 25 and 26 June 2019) under the same climatic conditions (no wind and no clouds). A total of 90 seedlings were collected and further analysed. The chlorophyll contents of the leaves of the seedlings were determined in August 2019 for 30 different seedlings in each stand due to difficulties in obtaining the instrumentation for measuring the chlorophyll content during the June 2019 field campaign. Laboratory measurements followed Pérez-Harguindeguy et al. (2013). Stem length, root length and leaf area of each seedling were measured on fresh materials. Three leaves for each seedling were fixed on a sheet and scanned using the Epson GT15000 scanner, and the leaf area (LA, cm^2) was calculated using the ImageJ software (Rasband 1997–2018). Fresh leaf mass saturated (LFMsat.) was calculated (after seven hours from the collection) and dry leaf mass (LDM), was calculated after drying leaves for 72 h at 60 °C until a stable weight was achieved. The leaf measures were used to evaluate the following plant functional traits: specific leaf area (SLA), leaf

Table 2. Description of the functional traits analysed (Pérez-Harguindeguy et al. 2013). §: the dimensionless value is converted into the total chlorophyll value (mg/cm2) using the conversion table https://www.atleaf.com/SPAD.

PFTs	Unit	Formula
Specific leaf area (SLA): is the area of a fresh leaf, divided by its oven-dry mass	cm^2/g	LA/LDM
Leaf dry matter content (LDMC): is the oven-dry mass of a leaf, divided by its water-saturated fresh mass	mg/g	LDM/LFMsat.
Leaf thickness (Lth)	-	1/SLA*LDMC
Leaf water content (LWC)	-	1000-LDMC
Leaf mass per area (LMA) is the oven-dry mass of a leaf, divided by its leaf area	g/cm^2	1/SLA
Leaf chlorophyll content (CHL)	mg/cm^2	§

dry matter content (LDMC), leaf thickness (Lth), leaf water content (LWC) and leaf mass per area (LMA) (Table 2).

It should be noted (Table 2) that some of the PFTs investigated express approximately the same concept providing redundant information (e.g., SLA/LMA or LDMC /LWC). Accordingly, we have decided to make a further selection considering three PFTs only, namely SLA, LDMC and Lth. A spreadsheet (Excel 2016) containing all the field and laboratory data was arranged and processed with XLSTAT 2020 3.1.3 software (Addinsoft 2005–2020). The data underpinning the analysis reported in this paper are deposited at "Mendeley Data" at https://doi.org/10.17632/6m2d4rxc7n.1. All the data were previously subjected to a normality check and then to a non-parametric comparison analysis, according to the Kruskal-Wallis method, with a paired comparison according to the Dunn method (with Bonferroni correction for significance, set at alpha = 0.05).

Results

Phytosociological survey

No published phytosociological relevés or previous syntaxonomic interpretation were available for the study area. All the three wood stands were assigned to a single association, *Roso arvensis-Quercetum cerridis* Ubaldi 2003 (syn: *Aremonio agrimonioidis-Quercetum cerridis* Blasi et al. 2005 ex Terzi et al. 2021). The phytosociological relevés (Table 3) showed some differences among the three sampled sites. Bosco della Ficora (BF) and Selva di Castiglione (SC) woods are characterised by a tree layer composed of *Quercus cerris* and *Carpinus betulus*. In central Europe, the abundance of *Carpinus betulus* is considered as favoured by clumsy cutting forest practices that lead the climax *Quercus petraea* and *Q. robur* forest to be fragmented and subsequently invaded by the hornbeam (Oberdorfer, 1992). In the case of our study area, the occurrence of *Carpinus betulus* in the dominant tree layer is a sign of moist and eutrophic soil conditions and a low degree of disturbance (Blasi et al. 2005). Indeed, the abundance of *Carpinus betulus* sharply decreases passing from northern Italy to southern Italy and this species normally does not participate in recolonisation stages after oak forest coppicing where it is substituted by *Fraxinus ornus, Acer opalus* subsp. *obtusatum*, *Corylus avellana* and *Ostrya carpinifolia* (the latter especially on limestone or calcareous marls). The BF and the SC woods show a mesophilous-microthermic aspect due to the presence of several *Fagetalia sylvaticae* species such as *Asperula taurina*, *Euphorbia amygdaloides*, *Crataegus laevigata*, *Cardamine bulbifera*, *Neottia nidus-avis*, *Euphorbia dulcis*, *Viola reichenbachiana* (Di Pietro et al. 2004). In one relevé from the BF wood, *Abies alba* also occurs. The SC stand exhibits abundance of species typical of the *Quercus cerris* undergrowth such as *Fragaria vesca*, *Festuca heterophylla*, *Allium pendulinum* and *Luzula forsteri*. Bosco di San Leo wood (BSL) acts as a more thermophilous *Q. cerris* wood, characterised by a thick secondary tree layer dominated by *Carpinus orientalis*, *Fraxinus ornus* and *Acer campestre* and a shrub layer dominated by *Cornus sanguinea*, *Ligustrum vulgare*, *Hedera helix* and *Rubus ulmifolius* (Taffetani et al. 2012). In this paper, we have classified the *Roso arvensis-Quercetum cerridis* in the alliance *Erythronio-Carpinion betuli* (*Fagetalia sylvaticae*) due to the occurrence of several *Fagetalia* species. However, this classification should be considered preliminary. A still unresolved debate is open on the classification of the *Quercus cerris* woods of the montane belt of central-southern Italy both at the class level (*Carpino-Fagetea* vs. *Quercetea pubescentis*) and at the alliance level (*Erythronio-Carpinion* vs. *Physospermo-Quercion cerridis* vs. *Melittio-Quercion frainetto* vs *Geranio striati-Fagion*). As this is not the paper in which to discuss this issue, we refer to the following bibliography: Biondi et al. 2002; Ubaldi 2003; Blasi et al. 2005; Di Pietro and Fascetti 2005; Rosati et al. 2005; Biondi et al. 2008, 2014; Mucina et al. 2017 and Di Pietro et al. 2020.

Syntaxonomic framework

QUERCO ROBORIS-FAGETEA SYLVATICAE Br.-Bl. et Vlieger in Vlieger 1937
FAGETALIA SYLVATICAE Pawlowski in Pawlowski, Sokolowski et Wallish 1928
Erythronio-Carpinion betuli (Horvat, 1958) Marincek in Wallnofer, Mucina et Grass 1993
Roso arvensis-Quercetum cerridis Ubaldi 2003

Table 3. *Roso arvensis-Quercetum cerridis* Ubaldi 2003.

Date	6/6/2019	6/6/2019	6/6/2019	6/6/2019	7/22/2010	7/22/2010	6/25/2019	
Forest Stand acronym	SC	SC	BF	BF	BSL	BSL	BSL	
Relevé number	1	2	3	4	5	6	7	
Aspect	-	-	NNW	E	N	NNE	NNE	
Elevation (m a.s.l.)	980	980	920	890	810	810	810	
Slope (%)	-	-	10	5	5	10	5	
Soil surface aspect, rocky outcrops (%)	-	-	-	-	-	-	-	
Soil surface aspect, stoniness (%)	-	-	-	-	1	-	-	
Upper tree layer cover (%)	65	65	55	70	65	70	50	
Upper tree layer height (m)	20	20	22	17	18	20	28	
Lower tree layer cover (%)	80	50	40	30	30	20	70	
Lower tree layer height (m)	2.5	2.5	3.4	5.6	4,5	6	4	
Shrubs layer cover (%)	-	-	-	10	50	40	90	
Shrubs layer height (%)	-	-	-	2	1.5	2	2	
Herbaceous layer cover (%)	60	40	95	75	30	40	10	
Area (m^2)	200	200	200	200	200	200	300	
Coverage (%)	100	80	90	90	100	100	100	
Species per relevé	26	26	35	26	22	22	24	frq.
***Roso arvensis-Quercetum cerridis* Ubaldi 2003**								
Rosa arvensis	1	+	1	1	2	+	1	V
Ligustrum vulgare	1	2	1	2	2	2	2	V
Lonicera caprifolium	2	2	2	2	+	+	.	V
Crataegus laevigata	+	.	.	.	.	.	1	II
***Erythronio-Carpinion betuli* (Horvat 1958) Marincek in Wallnofer, Mucina & Grass 1993**								
Carpinus betulus	4	3	2	2	.	+	.	IV
Primula vulgaris	+	1	+	.	1	+	.	IV
Cardamine bulbifera	2	2	+	+	.	.	.	III
Pulmonaria apennina	+	.	+	.	.	+	.	III
Viola odorata	.	.	.	.	+	.	.	I
Carex sylvatica	.	.	.	.	.	.	1	I
***Geranio versicoloris-Fagion* Gentile 1970**								
Cyclamen hederifolium	+	+	.	+	.	.	.	III
Allium pendulinum	2	1	+	.	.	.	.	III
Anemone apennina	+	+	+	+	.	.	.	III
Ranunculus lanuginosus	.	.	+	.	1	.	.	II
Geranium versicolor	.	.	+	.	.	.	.	I
Lathyrus venetus	.	.	.	+	.	.	.	I
***Fagetalia sylvaticae* Pawłowski in Pawłowski, Sokołowski & Wallisch 1928**								
Euphorbia amygdaloides	.	.	2	.	.	.	1	II
Euphorbia dulcis	.	.	+	+	.	.	.	II
Fraxinus excelsior	+							
Asperula taurina	.	.	1	.	.	.	.	I
Neottia nidus-avis	.	.	+	.	.	.	.	I
Mycelis muralis	.	+	.	.	.	.	.	I
Galium odoratum	.	.	.	.	.	+	.	I
Abies alba	.	.	.	1	.	.	.	I
***Quercetalia pubescenti-petraeae* Klika 1933**								
Brachypodium rupestre	1	1	2	1	.	.	+	IV
Sorbus torminalis	.	+	.	.	.	1	+	III
Cornus mas	.	.	1	+	.	.	1	III
Fraxinus ornus	.	.	.	+	.	1	1	III
Carpinus orientalis	.	.	.	.	1	2	2	III
Veronica chamaedrys	+	+	.	.	.	.	.	II
Aegonychon purpurocaeruleum	.	.	2	.	.	.	.	I
Helleborus foetidus	.	.	.	.	.	+	.	I
Scutellaria columnae	.	.	.	.	.	.	1	I
***Querco-Fagetea* Br.-Bl. & Vlieger in Vlieger 1937**								
Quercus cerris	4	3	3	4	4	4	4	V
Hedera helix	1	1	1	1	2	3	1	V
Rubus hirtus	+	1	2	3	+	+	.	V
Daphne laureola	1	+	+	.	+	+	.	IV
Acer campestre	.	.	2	2	2	1	3	IV
Ajuga reptans	+	1	.	.	+	.	1	III
Festuca heterophylla	2	2	1	+	.	.	.	III
Crataegus monogyna	.	.	1	2	+	.	+	III

Table 3. Continuation.

Date	6/6/2019	6/6/2019	6/6/2019	6/6/2019	7/22/2010	7/22/2010	6/25/2019	
Forest Stand acronym	SC	SC	BF	BF	BSL	BSL	BSL	
Relevé number	1	2	3	4	5	6	7	
Aspect	-	-	NNW	E	N	NNE	NNE	
Elevation (m a.s.l.)	980	980	920	890	810	810	810	
Slope (%)	-	-	10	5	5	10	5	
Soil surface aspect, rocky outcrops (%)	-	-	-	-	-	-	-	
Soil surface aspect, stoniness (%)	-	-	-	-	1	-	-	
Upper tree layer cover (%)	65	65	55	70	65	70	50	
Upper tree layer height (m)	20	20	22	17	18	20	28	
Lower tree layer cover (%)	80	50	40	30	30	20	70	
Lower tree layer height (m)	2.5	2.5	3.4	5.6	4,5	6	4	
Shrubs layer cover (%)	-	-	-	10	50	40	90	
Shrubs layer height (%)	-	-	-	2	1.5	2	2	
Herbaceous layer cover (%)	60	40	95	75	30	40	10	
Area (m^2)	200	200	200	200	200	200	300	
Coverage (%)	100	80	90	90	100	100	100	
Species per relevé	26	26	35	26	22	22	24	frq.
Fragaria vesca	2	2	+	.	.	.	.	III
Cruciata glabra	+	+	+	+	.	.	.	III
Luzula forsteri	1	1	.	.	+	.	.	III
Dioscorea communis	.	+	+	.	.	.	.	II
Digitalis micrantha	+	.	.	.	.	.	.	I
Lilium bulbiferum	.	1	.	.	.	.	.	I
Melica uniflora	.	.	.	+	.	.	.	I
Bromopsis ramosa	.	.	.	.	+	.	.	I
Campanula trachelium	.	.	.	.	.	.	1	I
Epipactis helleborine	.	.	.	.	.	.	+	I
Rhamno-Prunetea **Rivas Goday & Borja ex Tuxen 1962**								
Euonymus europaeus	.	.	1	1	+	1	.	III
Cornus sanguinea	.	.	.	.	3	2	1	III
Pyrus communis	.	.	+	+	2	.	.	III
Clematis vitalba	.	.	1	.	.	.	1	II
Prunus spinosa	.	.	.	.	1	+	.	II
Rubus umifolius	.	.	.	.	.	1	3	II
Ulmus minor	.	.	.	.	.	.	1	I
Other species								
Agrimonia eupatoria	.	.	.	+	+	+	.	III
Carex remota	.	.	.	.	1	.	1	II
Hieracium gr. murorum	+	.	.	.	.	.	.	I
Malva thuringiaca	.	.	+	.	.	.	.	I
Campanula glomerata	.	.	.	.	.	.	+	I
Dactylis glomerata	.	+	.	.	.	.	.	I
Astragalus glyciphyllos	.	.	+	.	.	.	.	I
Carex sylvatica	.	.	1	.	.	.	.	I
Arum italicum	.	.	.	+	.	.	.	I
Orobanche hederae	.	.	.	.	.	+	.	I

Pignatti-Ellenberg's indices

Pignatti-Ellenberg's indices calculated for each forest stand were found to be the following: the SC stand: L= 5.179, T= 5.885, U= 5.408 and N= 5.600. For the BF stand, they were L= 5.932, T= 6.590, U= 4.644 and N= 4.962. For the BSL stand, they were L= 5.627, T= 6.742, U= 4.185 and N=3.341. The comparison among stands showed for the SC stand, higher values for soil moisture (U) and nutrients (N) compared to those of the BF and the BSL stands. In contrast, the BF and the BSL stands exhibited higher values for temperature and light (T and L) (Fig. 2).

Bulk density and pH of soil

The bulk density is an indicator of soil compaction. The three sampled stands exhibit similar mean values of bulk density (p-value is not significant), although the BSL and the BF stands showed a wide range of values, whereas the SC stand showed a narrow range of values together with the presence of some outliers (Fig. 3). According to the Natural Resources Conservation Service (USDA-NRCS 2021), the values we have found are considered "ideal conditions for plant growth". Based on the classes of the pH soil described by the National Soil Survey Center

(1998), the SC stand is classified as “extremely acid”, with an average pH value of 4.5, the BF stand is classified “very strongly acid”, with an average value of 4.7 and the BSL stand is classified as “strongly acid”, with an average value of 5.6 (Fig. 3).

Morphological measurements

Seedling root lengths were found to be slightly shorter in the SC stand compared to the other two stands, whereas stem lengths were found to be slightly shorter for the

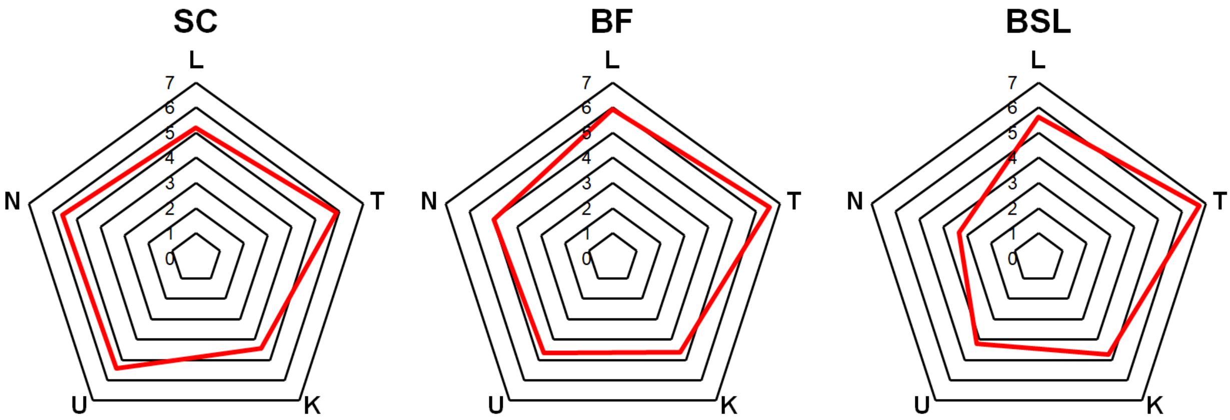

Figure 2. Ecograms for the three forest stands based on the average values for Pignatti-Ellenberg indices: L – light; T – temperature; K – continentality; U – soil moisture; N – soil nutrients.

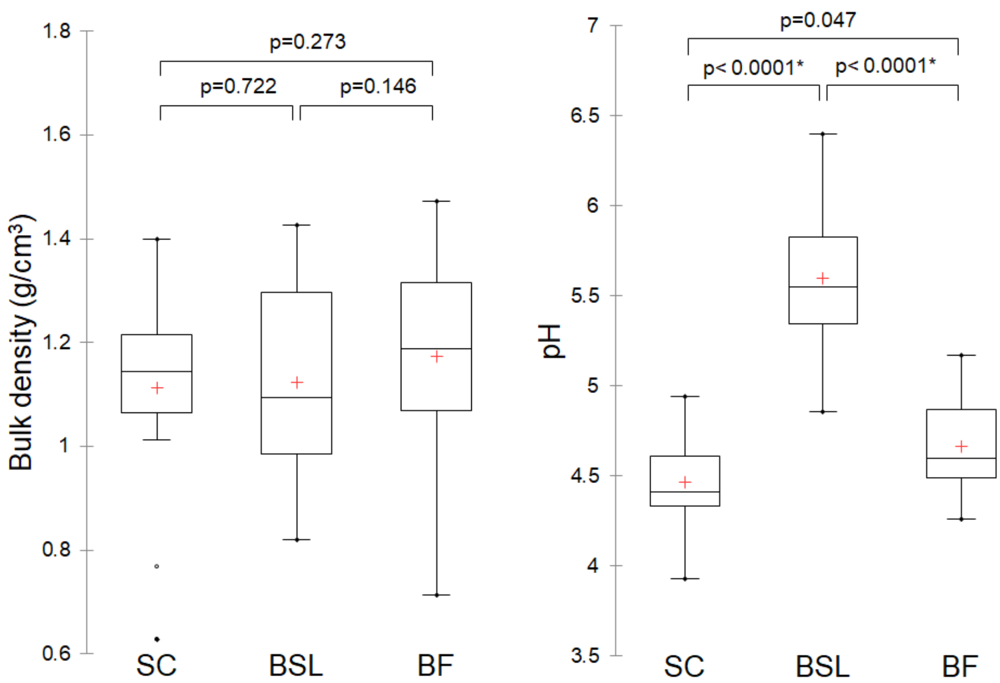

Figure 3. Bulk density and pH of soil in the three wood stands. Non-parametric comparison analysis (Kruskal-Wallis test) and pair comparison according to the Dunn method with Bonferroni correction for significance. Number of replicates for each stand: 30. Legend: red cross = mean; horizontal bar in the box = median; lower limit of the box = first quartile; upper limit of the box = third quartile; empty dots and stars outer the whiskers' bounds = outliers; filled diamonds = minimum and maximum values. Empty dots: values found in the [Q1 – 3 (Q3 - Q1); Q1 - 1.5 (Q3 - Q1)] interval or in the [Q3 + 1.5 (Q3 - Q1); Q3 + 3 (Q3 - Q1)] interval. See Appendix 1, Table A1 for the summary statistics.

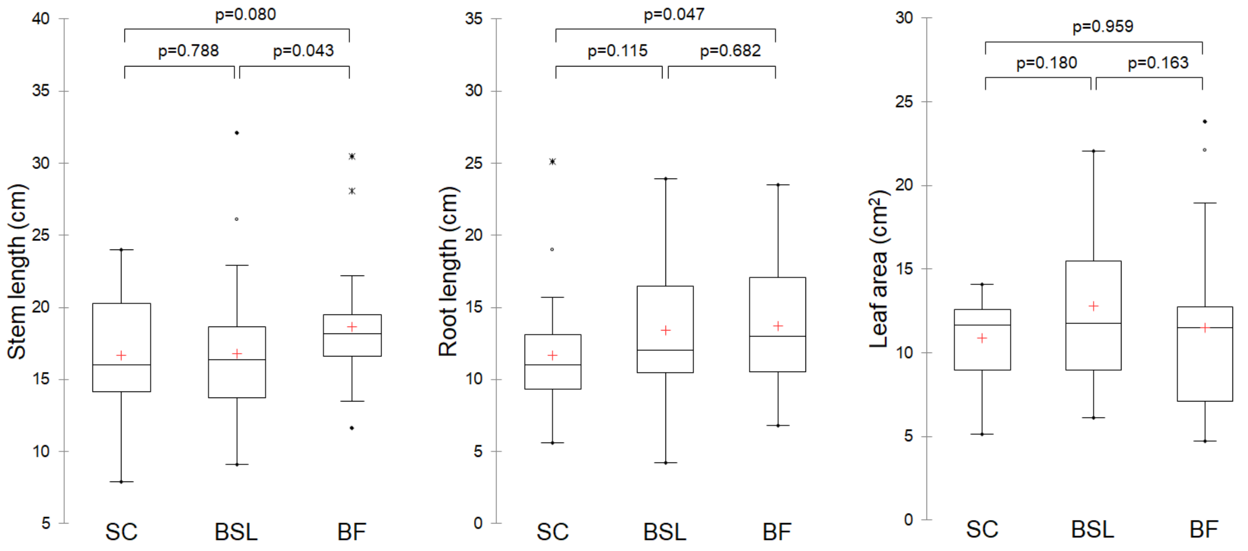

Figure 4. Stem length, root length and leaf area values in the three wood stands. Non-parametric comparison analysis (Kruskal-Wallis test) and pair comparison according to the Dunn method with Bonferroni correction for significance. Number of replicates for each stand: 30. Legend: red cross = mean; horizontal bar in the box = median; lower limit of the box = first quartile; upper limit of the box = third quartile; empty dots and stars outer the whiskers' bounds = outliers; filled diamonds = minimum and maximum values. Asterisks: values found outside the [Q1 - 3 (Q3- Q1); Q3 + 3 (Q3 - Q1)] interval. Empty dots: values found in the [Q1 – 3 (Q3 - Q1); Q1 - 1.5 (Q3 - Q1)] interval or in the [Q3 + 1.5 (Q3 - Q1); Q3 + 3 (Q3 - Q1)] interval. See Appendix 1, Table A2 for the summary statistics.

BSL stand. However, these differences in the mean values of the stem and root lengths turned out not to be statistically significant, with the p-value very close to the threshold (alpha=0.05, Fig. 4). Differences in the values of leaf area among the three stands investigated turned out to also be statistically insignificant (Fig. 4).

Plant functional traits

Specific leaf area (SLA) measurements showed the highest mean value in the SC stand (265.551), which differed significantly from the BF stand (215.078) and the BSL stand (205.224) (p=0.000 and p=0.0001 respectively), the values of the latter being very similar (p=0.508) (Fig. 5). Leaf dry matter content (LDMC) measurements showed that the mean value of the SC stand (339.652) differs significantly from those of the BF stand (407.723) and the BSL stand (416.036), with the SC displaying the lowest mean value (p=0.001) (Fig. 5). For Lth, the low values obtained for the SC stand (1.362), differed significantly from those obtained for the BF stand (1.983) and the BSL stand (2.301) (p=0.001) (Fig. 5).

Leaf chlorophyll content

The SC stand exhibited the highest average value for CHL (0.022 mg/cm^2). This value differs significantly from those displayed by the BSL (0.016 mg/cm^2) and BF stands (0.014 mg/cm^2) (Fig. 6).

Discussion

All three wood stands investigated were assigned to the same association, which is *Roso arvensis-Quercetum cerridis* Ubaldi 2003. Nevertheless, owing to their different silvicultural treatment and ecological features, the three forest stands exhibited clear floristic-coenological differences. The BSL stand is dominated by *Q. cerris* in the upper tree layer and *Carpinus orientalis* in the lower tree layer, and the coverage of the shrub layer was found to be very high (40–90%). The PEi values were high for T and L. The BF stand is characterised by a dominance of *Quercus cerris*, with *Carpinus betulus* having a subordinate role, and by a high coverage by the herb layer (75–90%). The PEi values were found to be high for T and L. The SC stand is characterised by the co-dominance of *Quercus cerris* and *Carpinus betulus* in the upper three layer and by an almost totally missing shrub layer. The PEi values were high for U and N and low for T and L.

The stem and root lengths and the leaf areas of the seedlings were found to be rather similar in the three wood stands. Even if the *Q. cerris* seedlings of the three wood stands investigated are phenotypically very close each other, they showed a different response to environmental conditions if the PFTs values are considered. In

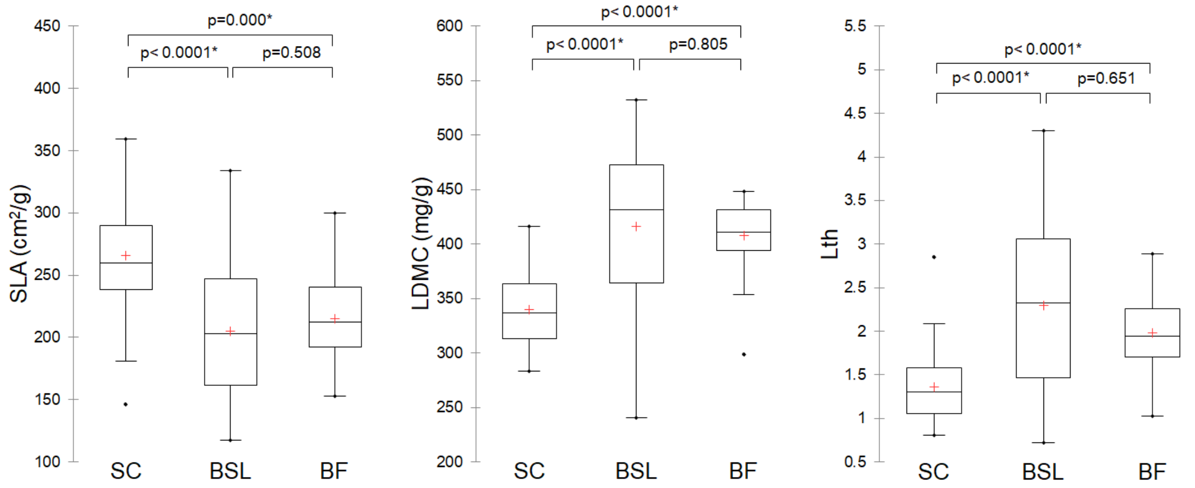

Figure 5. SLA, LDMC and Lth values in the three wood stands. Non-parametric comparison analysis (Kruskal-Wallis test) and pair comparison according to the Dunn method with Bonferroni correction for significance. Number of replicates for each stand: 30. Legend: red cross = mean; horizontal bar in the box = median; lower limit of the box = first quartile; upper limit of the box = third quartile; empty dots and stars outer the whiskers' bounds = outliers; filled diamonds = minimum and maximum values. Empty dots: values found in the [Q1 – 3 (Q3 - Q1); Q1 - 1.5 (Q3 - Q1)] interval or in the [Q3 + 1.5 (Q3 - Q1); Q3 + 3 (Q3 - Q1)] interval. See Appendix 1, Table A3 for the summary statistics.

the ecological literature, high values of SLA are normally related to environments characterised by being rich in nutrients and/or by shaded conditions. The higher values of SLA showed by the saplings of the SC stand compared to those of the BF and the BSL testify to the propensity of the SC seedlings for an investment in carbon oriented to a rapid growth of the saplings rather than to store material for longevity. High SLA values are related to a lower occurrence of dense tissues, which, as a negative counterpart, makes these seedlings more palatable to grazing herbivores. This negative effect is balanced by a more rapid production of new leaves, linked to a higher availability in nutrients, water, as suggested by the PEi values (see Fig. 3) (Wilson et al. 1999; Shipley et al. 2002; Wright et al. 2004; Pontes et al. 2007).

In both the BSL and the BF stands, the values of LDMC are higher than those measured in the SC, showing, therefore, for the seedlings of the BSL and the BF a greater propensity to longevity (Dijkstra and Lambers 1989; Niemann et al. 1992; Garnier and Laurent 1994; Kazakou et al. 2006). The low SLA and the high LDMC values in the BSL and the BF highlight different strategies compared to the SC stand. We could indirectly address these differences to a lower presence of resources for leaf development. The low Lth values of the SC stand are related to seedlings with thinner leaves. This is another PFT that identifies leaves with a greater propensity to grow. The Lth values are normally negatively correlated with photosynthesis and growth, while being positively correlated with leaf longevity and construction costs (Vile et al. 2005).

Considering that PFT values, such as SLA, LDMC and Lth, are in many cases used to estimate the growth rate of seedlings (Poorter and Garnier 1996; Wright and Westoby 2001), it can be assumed that the SC seedlings grow faster than those of the BF and the BSL. In broad terms, high values in SLA and low values in LDMC correspond to a rapid production of biomass, whereas the low values in SLA and high values in LDMC indicate the conservation of nutrients through long-lived leaves (Reich 1998; Garnier et al. 2001). Assuming that the SC stand seedlings benefit from a greater number of resources that lead these seedlings to exhibit higher SLA values, the next step could be making hypothesis on which kinds of resources are important. In this case, the response derives directly from the PEi, which indicates, for the SC stand, a higher availability of nutrients (N) and soil moisture (U). The flat geomorphological profile of the SC stand and the clayey substrate may play a crucial role in leading to a high degree of soil water retention. While refraining from drawing up a ranking of PEi by importance, we underline the effect that soil moisture can have for the deciduous forest communities occurring in Mediterranean or sub-Mediterranean environments, these latter being usually characterised by more or less prolonged dry periods during late spring and summer.

The pH of the soil, on the other hand, would seem to provide values in contrast, as it goes from 4.5 (SC) to 4.7

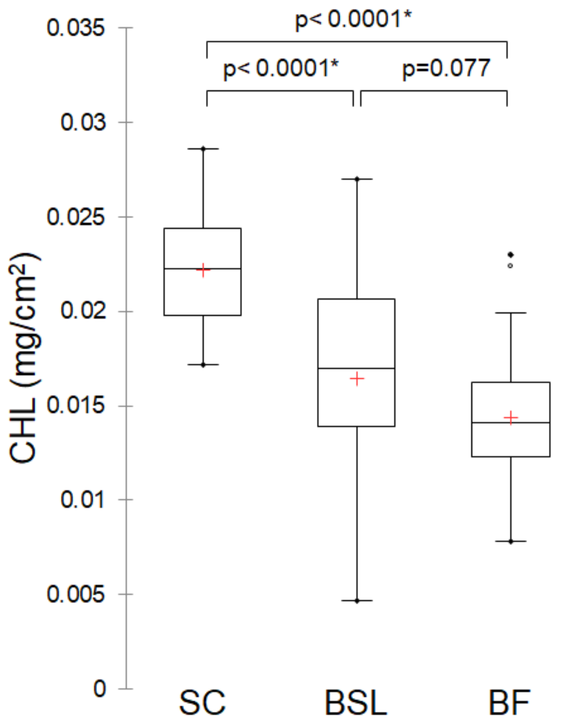

Figure 6. CHL values in the three wood stands. Non-parametric comparison analysis (Kruskal-Wallis test) and pair comparison according to the Dunn method with Bonferroni correction for significance. Number of replicates for each stand: 30. Legend: red cross = mean; horizontal bar in the box = median; lower limit of the box = first quartile; upper limit of the box = third quartile; empty dots and stars outer the whiskers' bounds = outliers; filled diamonds = minimum and maximum values. Empty dots: values found in the [Q1 – 3 (Q3 - Q1); Q1 - 1.5 (Q3 - Q1)] interval or in the [Q3 + 1.5 (Q3 - Q1); Q3 + 3 (Q3 - Q1)] interval. See Appendix 1, Table A4 for the summary statistics.

(BF) and 5.6 (BSL). These values are more consistent with a typical oligotrophic Quercetalia robori-petraea wood than with eutrophic *Quercus cerris* woods of *Erythronio-Carpinion*. Instead, the pH values of the BSL site were perfectly in line with this latter alliance. It would be interesting to understand if these low pH values of the SC and the BF could at least partially explain why the montane-belt *Quercus cerris* forests often give rise to almost monophytic communities, or in any case, communities lacking other species of oaks (see for example Di Pietro and Tondi 2005). It is possible that in addition to the altitude, the low soil pH values lead *Quercus cerris* to be less subjected to the competition from other oak species that are common in this area (e.g. *Q. pubescens* and *Q. frainetto*), whereas acidophilic oaks (e.g., *Q. petraea* and *Q. robur*) that potentially could take advantage from such low pH values are missing due to their rareness in southern Italy.

Another resource that could explain the high SLA values observed for the SC stand could be the light radiation. The PEi values showed a significant difference between the low value (5.19) of the SC stand and the values of the BF and the BSL stands (5.932 and 5.627, respectively).

Observing the headline of the phytosociological table, it emerges that the SC wood stand, while showing a high coverage index at the upper tree layer is characterised by an almost total lack of shrub layer. On the contrary, the BSL wood stand shows the highest cover indices for the shrub layer. It is possible, therefore, that in the SC population, the high coverage of the tree layer was much more effective in reducing the amount of light available for the herb layer than the dense shrub layer occurring in the BSL stand. Accordingly, the high Lth values observed in the BSL stand could be due to the low coverage value of the upper tree layer. In this case, a shrub layer, composed of prostrate species, such as *Hedera helix* and *Rubus ulmifolius* occurring in the BSL would not seem to represent an effective impediment to light in reaching the herb layer.

As far as values of CHL are concerned, these were found to be higher in the SC stand than in the other two stands. A greater quantity of chlorophyll favours a greater absorption capacity of solar radiation. Based on published studies (e.g., Curran et al. 1990; Filella et al. 1995; Gitelson et al. 2003) higher chlorophyll content suggests also a higher photosynthetic potential. This is in accordance with other PFT values observed in this study, such as the lower Lth values and with the higher soil moisture, which would turn out to be higher in the SC stand than in the other two stands (see Fig. 2). The soil moisture is one of the factors that is normally considered as influencing the photosynthetic potential (Zhang et al. 2011; Aref et al. 2013). All these results seem to agree that the seedlings of the SC stand, due to the lower light irradiance and higher soil moisture, are more prone to a rapid development than the seedlings of the BF and the BSL stands. The latter (BLS), growing on slightly drier substrates and greater light intensity, exhibits seedlings developing thicker leaves to cope with lower water availability and higher irradiance (Wright et al. 2004; Poorter et al. 2009).

Conclusions

In this study, phenotypic parameters, and some plant functional traits of *Quercus cerris* seedlings were tested for the first time in natural forest communities to highlight possible adaptation strategies to environmental conditions. The results do not show a significant link between phenotypic plasticity and environmental parameters. It emerged (at least in our case), that the phenotypic expression is not related with the growth strategy of the seedlings. Accordingly, the phenotypic plasticity of the *Quercus cerris* seedlings should not be considered a target element to detect the fitness to ensure the growth of the tree. On the contrary, PFT values allowed for highlighting significant differences between the three wood stands considered and for hypothesising possible strategies adopted by *Quercus cerris* in the first years after germination, depending on the environmental characteristics of the sites. Apparently, the PFT values evidenced that the seedlings of Selva di Castiglione (SC) were following a different development strategy than those of the Bosco della Ficora (BF) and Bosco di San Leo (BSL). Thus, the SC seedlings strategy, expressed by higher values of SLA and CHL and lower values of LDMC and Lth leads to the production of thinner leaves and testifies a greater investment in carbon aimed to a rapid growth of the seedling. The presence of more favorable conditions in the SC stand for the development of the seedlings is supported by the Pignatti-Ellenberg index (PEi) values, which confirm for this site a greater availability of nutrients and soil humidity.

Beyond contributing to a relatively still poor national PFTs database with the first data of in situ PFTs from southern Italy regarding a species of enormous territorial value such as *Quercus cerris*, this study emphasises the importance of investigating on the relationships between young plant development and environmental conditions. The in situ studies on plant functional traits of seedlings of forest species, providing crucial information on their ecology, may assume a great importance to address forest management practices. This information is likely to prove useful in the near future, precisely in the light of recent Italian and European policies linked to the containment of global warming. The guidelines for the "urban and extra-urban forest plan" of the National Recovery and Resilience Plan (Mission 2; Component 4; Investment 3.1) recently published by the Italian Ecological transition Ministry require specific references to natural forest communities and native woody flora to propose models of new urban forests that are consistent with the environmental characteristics of the sites and with the natural potential vegetation types. Yet, the new EU forestry strategy, which plans to plant 3 billion more trees in the European territory by 2030, can become an opportunity to apply the principles of ecology and vegetation science to sustainable land management.

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Appendix - Summary statistics

Table A1. Summary statistics for Bulk density and pH.

	Bulk density (g/cm^3)				pH			
Stand	*Minimum*	*Maximum*	*Average*	*Standard deviation*	*Minimum*	*Maximum*	*Average*	*Standard deviation*
SC	0.627	1.399	1.113	0.181	3.93	4.94	4.469	0.267
BSL	0.820	1.425	1.123	0.180	4.86	6.40	5.599	0.360
BF	0.713	1.472	1.174	0.181	4.26	5.17	4.666	0.233

Table A2. Summary statistics for Stem length, Root length and Leaf area.

	Stem length (cm)				Root length (cm)				Leaf area (cm^2)			
Stand	*Minimum*	*Maximum*	*Average*	*Standard deviation*	*Minimum*	*Maximum*	*Average*	*Standard deviation*	*Minimum*	*Maximum*	*Average*	*Standard deviation*
SC	7.900	24.000	16.697	4.257	5.600	25.100	11.650	3.918	5.114	14.092	10.859	2.448
BSL	9.100	32.100	16.780	4.825	4.200	23.900	13.397	5.102	6.124	22.033	12.799	4.272
BF	11.600	30.500	18.633	3.698	6.800	23.500	13.707	4.512	4.710	23.806	11.495	4.742

Table A3. Summary statistics for the PFTs Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC) and Leaf thickness (Lth).

	SLA (cm^2/g)				LDMC (mg/g)				Lth			
Stand	*Minimum*	*Maximum*	*Average*	*Standard deviation*	*Minimum*	*Maximum*	*Average*	*Standard deviation*	*Minimum*	*Maximum*	*Average*	*Standard deviation*
SC	146.135	359.631	265.551	51.847	283.037	416.391	339.652	37.281	0.806	2.854	1.362	0.431
BSL	117.310	334.237	205.224	55.258	240.648	532.341	416.036	74.523	0.720	4.306	2.301	0.939
BF	152.591	299.809	215.078	34.135	298.322	448.317	407.723	31.926	1.022	2.887	1.983	0.419

Table A4. Summary statistics for the chlorophyll content (CHL).

	CHL (mg/cm^2)			
Stand	*Minimum*	*Maximum*	*Average*	*Standard deviation*
SC	0.017	0.029	0.022	0.003
BSL	0.005	0.027	0.016	0.006
BF	0.008	0.023	0.014	0.004