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Original article

# Soil biota effects on clonal growth and flowering in the forest herb *Stachys sylvatica*

Eduardo de la Peña [a,b,*], Dries Bonte [a]

[a] Terrestrial Ecology Unit, Department of Biology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium
[b] Laboratory for Forestry, Faculty of Bioscience Engineering, Geraardsbergse Steenweg 2679090 Melle-Gontrode, Belgium

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ABSTRACT

The composition of a soil community can vary drastically at extremely short distances. Therefore, plants from any given population can be expected to experience strong differences in belowground biotic interactions. Although it is well recognized that the soil biota plays a significant role in the structure and dynamics of plant communities, plastic responses in growth strategies as a function of soil biotic interactions have received little attention. In this study, we question whether the biotic soil context from two forest associated contrasting environments (the forest understory and the hedgerows) determines the balance between clonal growth and flowering of the perennial *Stachys sylvatica*. Using artificial soils, we compared the growth responses of this species following inoculation with the mycorrhizal and microbial community extracted either from rhizospheric soil of the forest understory or from the hedgerows. The microbial context had a strong effect on plant functional traits, determining the production of runners and inflorescences. Plants inoculated with the hedgerow community had a greater biomass, larger number of runners, and lower resource investment in flower production than was seen in plants inoculated with the understory microbial community. The obtained results illustrate that belowground biotic interactions are essential to understand basic plastic growth responses determinant for plant establishment and survival. The interactions with microbial communities from two contrasting habitats resulted in two different, and presumably adaptive, growth strategies that were optimal for the conditions prevalent in the environments compared; and they are as such an essential factor to understand plant–plant, plant–animal interactions and the dispersal capacities of clonal plants.



## 1. Introduction

Variation in abiotic soil attributes has long been acknowledged as one determining factor that shapes plant community structure and dynamics (Maestre et al., 2006; Ehrenfeld et al., 2005; Hutchings et al., 2003; Kleb and Wilson, 1997). Nevertheless, during the last decade, a considerable body of literature has also highlighted the influential role of soil biota on the functioning of the plant community (Bever, 2003; Van der Putten et al., 2003). Plants selectively affect the soil biota associated with their rhizosphere, which creates feedback interactions between plants and soil (Bever et al., 1997; Reynolds et al., 2003; van der Putten, 2003). However, the distribution of soil biota displays distinct spatial patterns that vary at different scales, ranging from a few centimeters to the landscape level (Ettema and Wardle, 2002). Consequently, depending on the location in which the plant grows pronounced differences can exist in the belowground biotic interactions that plants experience and therefore, resulting in different feedback. Paradoxically, although it is well recognized that the interaction between plants and different members of the soil biota may affect important life history traits, the plastic responses in plant growth in relation to contrasting soil biotic environments have received scarce attention. Understanding the roles of soil biota on plant functional architecture is not only necessary to comprehend the interactions of individual plants with other members of the plant community and trophic levels (Wildová et al. 2007); but also from a spatial ecological perspective, to predict dispersal and plant population establishment and persistence (Gross et al., 2009; Wildová et al. 2007).

Clonality is a widespread trait in the plant kingdom (Mogie and Hutchings, 1990). Clonal growth is characterized by the spatial extension of an individual plant through repeated formation of new modules, which are morphologically connected and physiologically

* Corresponding author. Terrestrial Ecology Unit, Department of Biology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium. Tel.: +32 499161540.
*E-mail address:* eduardo.delapena@ugent.be (E. de la Peña).

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integrated through spacers (such as runners, rhizomes or roots) (Alpert and Stuefer, 1997). Clonal plants can perceive differences in habitat quality and establish ramets that exploit the best landscape locations (Day et al., 2003; Kleijn and van Groenendael, 1999; Wijesinghe and Hutchings, 1999; Jackson et al., 1990). The partitioning into functional modules allows clonal plants to respond to environmental changes by adapting plant growth responses to cope optimally with the surrounding environment; for instance, to avoid inter- and intra-specific competition, to optimally exploit resources, or to avoid natural enemies (Wijesinghe et al., 2005; Day et al., 2003; Wennström, 1999; Jackson et al., 1990). Clonal plant architecture (at least in terms of the number and the length of stolons produced) can be further conditioned by interactions with pathogens (Pan and Clay, 2003, 2002; D'Herterefeldt & Van der Putten, 1998).

At the landscape level, different plant communities create different biotic soil environments that might directly impinge on the plant growth of the members of the community. This possibility is not only interesting with respect to understanding plant responses at the individual level, but also has repercussions for forecasting population establishment and dynamics. However, a vegetative mode of propagation and spreading in clonal plants does not preclude also reproducing sexually by the production of flowers. Consequently is also important to understand which environmental factors in the soil trigger clonal growth (i.e. production of stolons) or flowering since there are different costs according to each type of growth mode (Hesse et al., 2008; Honnay, 2008).

Forest associated species are a good example of plants coping with extreme environmental gradients. These plants are exposed to strong changes in both biotic and abiotic conditions that act both above and belowground (e.g., solar radiation, water and nutrient availability, and herbivory, among others). For instance, many herbaceous forest species are able to colonize and establish viable populations in the understory of the forest and in adjacent hedgerows, both of which act as important species reservoirs (Wehling and Dieckman, 2009; Endels et al., 2004). However, the soil communities associated with mature forests, understory plantations or hedgerows are expected to be quite different as a result of the varied composition of the plant community and the different rates of deposition of litter and litter decomposition (Saetre and Baath, 2000; Priha and Sloander, 1999; Saetre, 1999).

One plant that displays this variation in small-scale distribution is *Stachys sylvatica* L., a perennial plant that is common in areas with intermediate light exposure and relatively high levels of soil humidity. It occurs in a large range of habitats, including open forests, forest verges, roadsides, and riversides (Van Landuyt et al., 2006). This species grows clonally, by means of runners, but it also produces conspicuous inflorescences with numerous nectar-producing flowers. *S. sylvatica* is a mycorrhizal plant that can cope with a wide range of forest related environments and consequently, the environmental (biotic and abiotic) conditions in which it grows can be extremely contrasting. Nevertheless, the consequences of growing in different soil environments and the effect of mycorrhizal colonization on clonal growth have not yet been studied.

The purpose of the present study was therefore to determine whether a clonal plant actually does modify its basic plant functional traits in response to soil biotic interactions. We hypothesized that differences in the belowground microbial composition in mature forests versus hedgerows would affect growth strategies of the clonal forest plant, *S. sylvatica*. We specifically hypothesized that soil biotic interactions might act upon two fundamental growth traits: vegetative growth (i.e., the production of stolons) and sexual reproduction (i.e., flowering). In order to test this hypothesis, we examined the effects of microbial biota extracted from these two contrasting forest environments on clonal growth and sexual reproduction in *S. sylvatica*.

## 2. Material and methods

### 2.1. Study site and collection of soil samples

Soil samples were collected in the *Heidebos* Nature Reserve. The Heidebos is a typical mosaic of natural and semi-natural woods and exploited agricultural landscape in the East of Flanders (Belgium). Although there are a wide variety of woodland types at this site, the most natural formations consist of open forests dominated by *Betula alba* and *Quercus robur* on the poorest soils, with a number of relatively large patches of *Fagus sylvatica* at more humid places. This type of formation corresponds with the characteristic vegetation for this latitude, soil and climatic conditions (Stortelder et al., 1999).

We collected soil samples in May 2008 from the understory of the forest (coordinates: 51°10′47.75N, 3°53′52.53E) and from adjacent hedgerows. Hedgerow sites were dominated by a mixture of typical shrub species such as *Rubus* spp. and *Sambucus nigra*, with a herbal community that included *S. sylvatica*, *Primula elatior*, *Ajuga reptans*, *Urtica dioica*, *Galium aparine*, *Silene dioica*, *Agrostis stolonifera*; unvegetated soil was covered mainly by mosses. We selected four hedgerows, separated at least 500 m from each other, and we took 10 soil samples at each of the four sites where *S. sylvatica* occurred. Each sub-sample consisted of 10 $cm^3$of rhizospheric soils. The mean plant coverage in the hedgerow sites was ca. 65% (with only 5% covered by *S. sylvatica*). The same procedure was followed for samples from the forest understory. In this case, we also selected four sites where *S. sylvatica* was present. The four sites were separated at least 500 m from each other and as in the case of hedgerow samples, we took 10 sub-samples. Plant coverage in these sites was also assessed. Forest understory sites were mainly dominated by grasses. These mostly were *Carex sylvatica*; *Dechampsia* spp., and *Poa* spp.. Plant coverage in the forest understory was near 30% (with 8% being *S. sylvatica* coverage).

### 2.2. Preparation of inocula

Once in the laboratory, sub-samples from each site and habitat type were manually mixed and sieved to remove large pieces of roots and stones. Afterward, 5 kg of each sample were mixed with 5 l of tap water in sterile plastic containers. The suspension was thoroughly mixed and left to incubate overnight. The next day, the soil–water suspension was divided into two fractions: one fraction was sieved successively through a series of sieves: 100 μm, 75 μm, and 40 μm mesh. This was done to extract the mycorrhizal community of the soil that we retained for further inoculation in experimental pots (see further). The other fraction was sieved only through a 40 μm, with this filtration we extracted the microbial community (Kardol et al., 2007). Sub-samples of 20 ml were taken from each of the extracts and inspected with the aid of a stereo microscope. If nematodes or other fauna were detected, the extracts were re-filtrated until no fauna was observed in the filtrate. Afterward, both fractions (i.e. mycorrhizal spores and the microbial fraction) were mixed and half of the resulting suspension from the soil from each type of site was autoclaved.

### 2.3. Characterization of mycorrhizal community

Twenty grams of soil from each sample were resuspended in 150 ml of water, stirred vigorously and sieved using 100, 75, 40 μm sieves as advised in An et al. (1990). The retained fraction on each sieve was filtered onto a filter paper. Spores were inspected and counted using a stereo microscope and then identified based on morphological traits (i.e. spore colour, size, surface and wall structures) with the descriptions provided by the International collection of vesicular and arbuscular mycorrhizal fungi (http://

invam.caf.wvu.edu). The rhizosphere soils contained an abundance of AMF spores that ranged between 49 and 127 spores per ml of soil. Rhizosphere soils coming from the forest understory had slightly lower densities than soils from the hedgerow, although these differences were not significant (data not shown). Within the AMF community extracted, 71% of the spores/sporocarps observed had a diameter of less than 70 μm (between 70 and 40 μm). Only 4% of spores had a diameter larger than 100 μm. Glomus was the dominant genus (75% of the detected spores) with *G. intraradices*, *G. aggregatum* and *G epigaeus* being the most frequent species. Spores of *Acaulospora* and *Scutellospora* were also detected. For these two genera the most dominant species corresponded to *A. spinosa* and *S. castanea* respectively. A relative high percentage of spores (i.e. 7%) could not be identified. No differences in the composition of the mycorrhizal community were observed between samples coming from the different forest environments.

### 2.4. Experimental set-up

Seeds of *S. sylvatica* were collected from natural stock populations and were germinated inside plastic boxes containing a mixture of 50% white sterile sand and 50% of sterile compost (both autoclaved 1 h at 120 °C. The germination trays were placed in a growth chamber at 22 °C and with additional illumination with a day/night regime of 14/10 h.

Plastic 1.5 l pots were filled with 1500 ml of a 3:1 v/v mixture of autoclaved commercial white river sand (Hubo ©) and autoclaved commercial garden soil. This type of mixture was chosen to standardize soil conditions since the soil composition was different in hedgerows and forest understory. After seed germination, two week-old seedlings were transferred to each pot. One week after seedling establishment in the pot, plants were inoculated with 80 ml of sterilized or unsterilized soil filtrates from the hedgerow soil or the forest understory. In both cases inocula contained a rate of 80 mycorrhizal spores per ml. This volume of inocula was chosen because in previous experiments such inoculation allowed for rapid and consistent colonization of plants roots; and also to avoid eventual changes in the nutrient content of the soil due to different abiotic soil characteristics from the different soil origins. In total, plants were inoculated with four types of filtrates: forest understory, sterilized forest understory, hedgerow, and sterilized hedgerow. The experiment compared the effect of origin (hedgerow vs. forest understory) and inoculation (sterilized inoculums vs. unsterilized). With this set-up, if changes in plants responses are due to the biotic component, they should only be prevalent in the pots inoculated with soil biota (i.e. unsterilized soil filtrates). Eighteen replicates were prepared per soil treatment. Pots were randomized on the bench of a growth chamber. Plants were left to grow under artificial illumination for 28 weeks. All plants were introduced to a cold chamber at 8 °C for three weeks and with a winter illumination regime (10/14 light/dark) one month after planting to induce flowering. After this "cold period", growth conditions were reset to summer conditions (14/10 light/dark regime with supplementary illumination efflux of 250 mol $m^{-2}\,h^{-1}$ at 22 °C). This illumination set-up resembles light conditions in open forest spots in the field. All pots were watered every 2 days with 50 ml of demineralized water, and fertilized every 3 weeks with 50 ml of a commercial fertilizer solution (COMPO ®, NPK 16–18–25) at the rate recommended by the manufacturer (1 g/l).

### 2.5. Harvest and data collection

Formation of inflorescences and the number of flowers/inflorescence was monitored and recorded on weekly basis. At harvest, the number of runners (stolons) produced and the length of the runners were measured. The dry weights of shoots and roots were measured after 48 h of drying in a 70 °C oven. Soil from 4 pots selected at random from each inoculated treatment was also analyzed at harvest.

A sub-sample from each root system was taken (including those plants inoculated with sterilized soil inocula to control for possible contaminations), cut into 1 cm pieces and stained with ink (Blue Quink, Parker) to estimate the percentage of mycorrhizal colonization in the roots, following a modification of a previously published protocol (Vierheilig et al., 1998). Root colonization was estimated using a stereoscopic microscope (Leica MZ 8) and the grid-line intersect method (Giovanetti and Mosse, 1980). For each plant, 50 root fragments were examined.

### 2.6. Statistical analysis

Normality and homogeneity of the variances were checked for all variables with Kolmogorov–Smirnov and with Levene's test, respectively. Variables were log (X + 1) transformed, when needed to meet ANOVA model assumptions (this was the case for the *number of inflorescences per plant*). A two factor ANOVA was performed to analyze the data: origin (hedgerow vs. forest) and inoculation (sterile soil inoculum vs. unsterilized soil inoculum). When ANOVA assumptions were not fulfilled, which occurred for the *number of runners per plant* and *mycorrhizal colonization*, we used a non-parametric Mann–Whitney test. Using this test, we performed multiple pair-wise comparisons (e.g. hedgerow vs. forest understory or hedgerow vs. and so for) and therefore we applied a conservative Bonferroni correction for multiple comparisons (i.e. only true significance at $p \leq 0.0125$).

## 3. Results

Overall, the inoculation with unsterilized soil had a positive effect on the biomass of *S. sylvatica* (Table 1, Fig. 1A–C). For all biomass (i.e. aboveground, belowground and total dry biomass), there were also differences according to the origin of the soil inocula (hedgerow vs. forest) (Table 1). The interaction between origin and inoculum was significant for below dry biomass and total dry biomass, which indicated that the effect of inoculation was dependent on the origin of the inoculums (forest understory vs. hedgerow) (Table 1). In this sense, the beneficial effect on plant growth was more pronounced in plants inoculated with understory soil biota than with hedgerow biota (Fig. 1).

The number of runners increased in plants inoculated with unsterilized soil suspension extracted from hedgerows (Fig. 2A) compared to the numbers for plants inoculated with sterilized inocula ($Z = -3.52$, $p = 0.00066$) or with inocula extracted from the forest understory ($Z = -3.52$; $p = 0.0001$). Runner length was similar for all soil treatments (Fig. 2B, Table 1). Runner weight did not differ across experimental treatments (Fig. 2C, Table 1).

Plants inoculated with unsterilized soil filtrates had a greater number of inflorescences than those inoculated with sterilized soil ($F_{1,68} = 4.78$; $p = 0.032$; Table 1). For this variable, the interaction between origin and inoculation was also significant ($F_{1,68} = 4.493$; $p = 0.038$; Table 1), which indicated that the effect of soil biota (inocula) was not the same between the two compared sites (hedgerow vs. forest understory). The greatest number of inflorescences was observed in plants inoculated with the microbial community extracted from the forest understory (Fig. 3A). No effect was seen in any treatment for the number of flowers per inflorescence (Fig. 3B, Table 1). The quantity of dry biomass allocated per inflorescence changed according to inoculation; plants inoculated with soil biota isolated from the forest understory had larger inflorescences than in any other treatment (Fig. 3C). So not only these plants produced more inflorescences, but also plants invested

**Table 1**
Summary of a two-way ANOVA performed for different growth variables of *Stachys sylvatica* using origin (with two levels hedgerow and forest understory) and inoculation (unsterilized or sterilized soil inoculums) as main factors. Bold letter types indicate a significant effect for the corresponding factor.

| | No. of inflorescences | | No. of flowers/ inflorescences | | Length of runners | | Inflorescence biomass | | Runner weight | | Ratio Inflorescence/ runner weight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factors | F | P | F | P | F | P | F | P | F | P | F | P |
| **O**rigin | 3.08 | 0.083 | 1.55 | 0.217 | 0.479 | 0.491 | 3.312 | 0.073 | 0.180 | 0.673 | 0.828 | 0.336 |
| **I**noculation | 4.781 | **0.032** | 1.23 | 0.270 | 2.875 | 0.094 | 5.821 | **0.019** | 2.949 | 0.090 | 0.27 | 0.888 |
| O × I | 4.493 | **0.038** | 0.021 | 0.886 | 0.479 | 0.491 | 7.883 | **0.007** | 0.019 | 0.664 | 7.364 | **0.008** |

| | Aboveground Biomass | | Belowground biomass | | Total biomass | |
|---|---|---|---|---|---|---|
| Factors | F | P | F | P | F | P |
| **O**rigin | 11.68 | **0.001** | 8.15 | **0.006** | 13.10 | **0.001** |
| **I**noculation | 31.89 | **0.0001** | 52.64 | **0.001** | 57.86 | **0.0001** |
| O × I | 1.55 | 0.218 | 7.64 | **0.007** | 6.20 | **0.015** |

relatively more biomass per inflorescence than in any other treatment (Fig. 3C). This differential allocation of resources can be seen when the ratio inflorescence/runner weight is compared (Fig. 3D). Values closer to 1 indicate a higher biomass allocation to inflorescences than to runners.

No differences in percentage of colonization were observed between plants inoculated with unsterilized inocula ($F_{1,34} = 1.55$; $p = 0.22$; Fig. 4). Mycorrhiza colonization was not observed in plants growing in sterilized soil.

Analysis of soil samples in the experimental pots did not reveal any differences in any soil parameters analyzed (Table 2).

## 4. Discussion

In the present study, the microbial differences in two contrasting forest habitats determined the balance between vegetative and sexual reproduction in the clonal plant *S. sylvatica*. According to expectations from adaptive plasticity, plants invested more in clonal growth when grown with a microbial community from the competitive hedgerow environment, and more into flowering when grown with the microbial community from the forest understory.

An emergent body of research in terrestrial ecology is now investigating plant–soil feedback, demonstrating that the interaction between plants and soil biota is not unidirectional and that it influences the performance and competitive ability of plant species and their offspring (Reynolds et al., 2003; van der Putten, 2003). Most of these studies have focused on long-term (e.g., primary and secondary succession, invasion) or community level processes (e.g., competition, facilitation) (see reviews by Kulmatiski et al., 2008). In contrast, the short-term effects of soil biota on plant individual responses have received less attention. Our results point out that the differences in the microbial communities associated with two contrasting forest habitats produce shifts in vegetative/reproduction balance that may determine a plant's success in establishing a viable population.

Shifts in a number of specific environmental factors, such as nutrient availability, soil moisture, photoperiod, can trigger plants to either continue vegetative growth or to become sexually

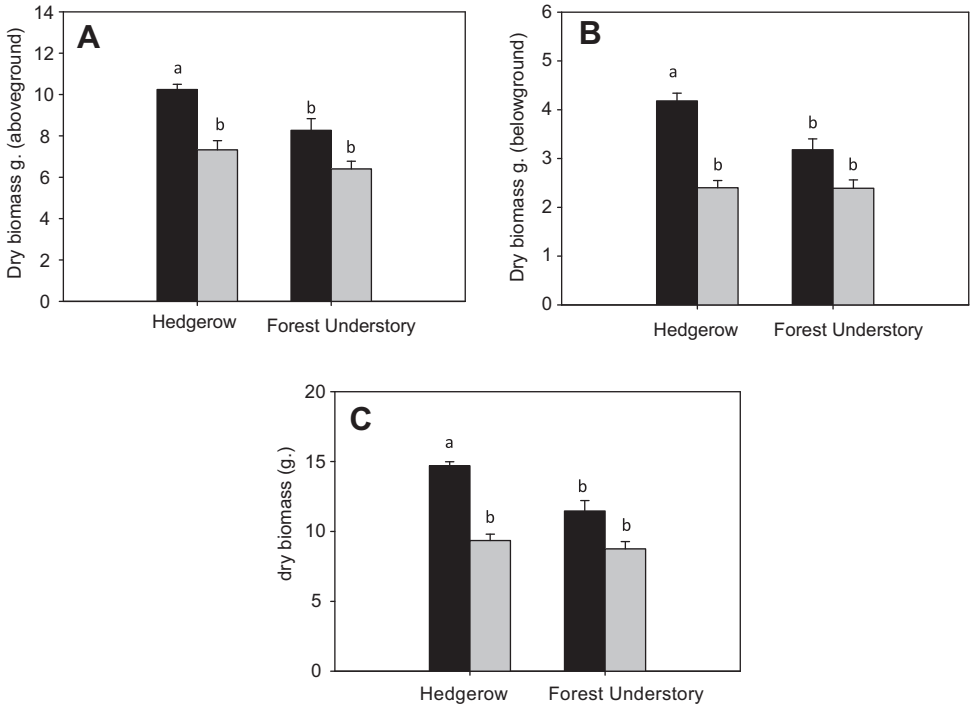


**Fig. 1.** (A) Aboveground dry biomass (mean ± SE), (B) belowground biomass (mean ± SE), (C) total dry biomass (mean ± SE) of *S. sylvatica* grown in soil inoculated with soil biota (black bars) or sterilized inocula (Gy bar) extracted from hedgerow or forest understory soil. Different letters indicate significant differences according to post-hoc Tukey's test ($p \leq 0.05$).

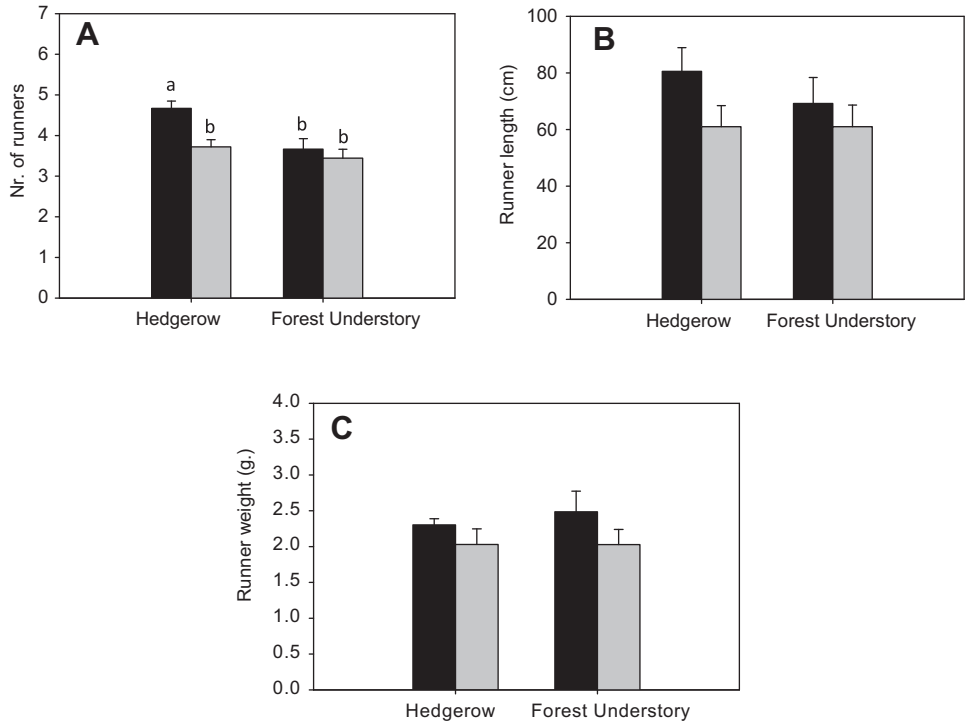


**Fig. 2.** (A) Number of runners (mean ± SE), (B) length of runners (mean ± SE) and (C) runner weiight of *S. sylvatica* grown in soil inoculated with soil biota (black bars) or sterilized inocula (Gy bar) extracted from hedgerow or forest understory soil. Different letters indicate significant differences after Mann–Whitney pair-wise comparisons ($p \leq 0.0125$).

reproductive. Such shifts in the vegetative growth/sexual reproduction have important consequences for the structure and dynamics of the plant community (Honnay, 2008). Ultimately, at the population level, the performance of any given species within a community will be affected not only by factors shaping growth, biomass allocation, and competition, but also by traits that condition the reproduction and spread of that species (Brewer et al., 1998). While the impact of soil nutrient conditions on plant growth architecture (Hutchings et al., 2003; Cahill and Casper, 1999; Birch and Hutchings, 1994) have been well addressed, the role therein of soil biota is usually overlooked and only a few papers have addressed the question (Bai et al., 2009; Cheplick, 2004; Pan and

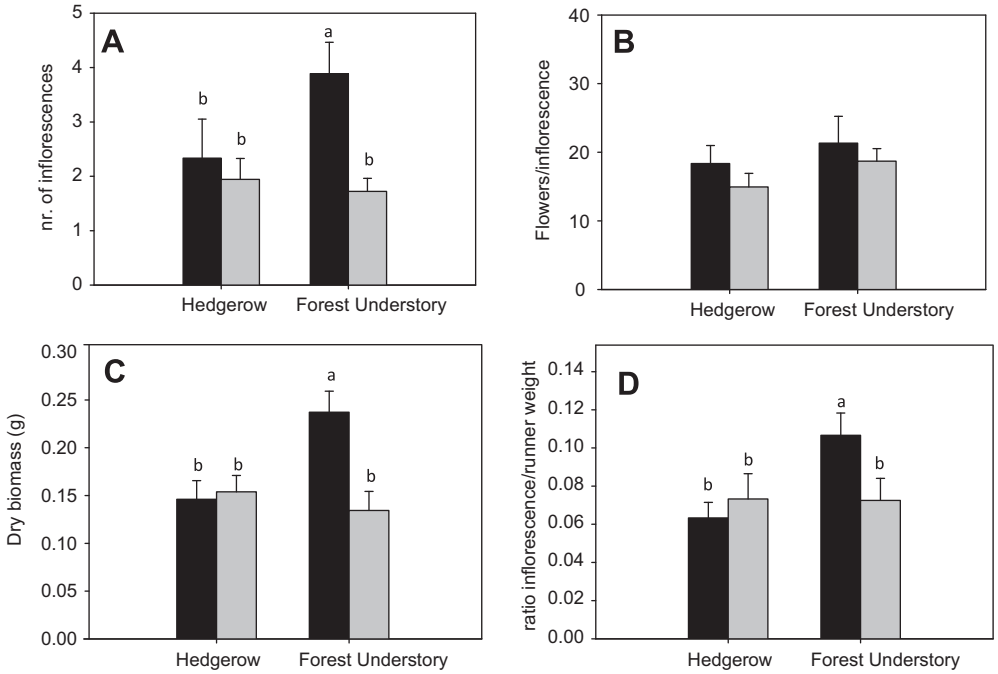


**Fig. 3.** (A) Number of inflorescences (mean ± SE), (B) number of flowers per inflorescence (mean ± SE) (C) biomass allocated per inflorescence and (D) ratio of biomass allocation to inflorescences and runner of *S. sylvatica* grown in soil inoculated with soil biota (black bars) or sterilized inocula (Gy bar) extracted from hedgerow or forest understory soil. Different letters indicate significant differences according to post-hoc Tukey's test ($p \leq 0.05$).

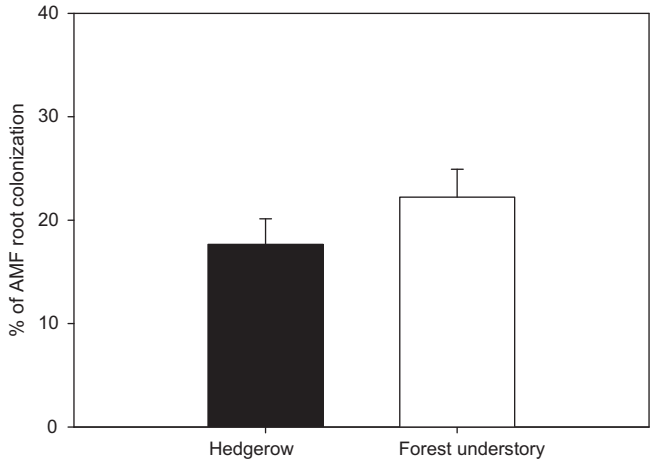


**Fig. 4.** Percentage (mean ± SE) of arbuscular mycorrhizal fungi colonization in roots of *Stachys sylvatica* inoculated with the microbial community extracted from the soil of the hedgerow or forest.

Clay, 2003; Streitwolf-Engel, 2001; D'hertefeldt and Van Der Putten, 1998). *S. sylvatica* showed an enhanced flower production (i.e. with a higher no. of inflorescences and a higher mean biomass per inflorescence) when inoculated with the microbial community isolated from forest understory soil, while inoculation with hedgerow microbes resulted in a higher number of runners per plant and fewer flowers. The higher investment in flowers in the forest understory may have consequences on overall population dynamics. *S. sylvatica* is a particularly attractive plant for pollinators (Comba et al., 1999). Therefore, an increase in the number of flowers may result in increased seed production and dispersal and, ultimately, in a pronounced shift from short to long distance dispersal events Müller-Landau et al. 2003.

Several studies suggest that clonal plants tend to allocate more resources to vegetative growth under sub-optimal or stress conditions Eriksson, 1986; Cook, 1985; Abrahamson, 1980, while other studies point in the opposite direction and indicate increased levels of vegetative growth under optimal conditions (Gardner and Mangel, 1999). These contrasting responses in the vegetative vs. sexual investment by plants might also be explained by other environmental factors, such as herbivory, light resources, plant age, and as pointed out before, resource availability (Bai et al., 2009; Van Kleunen and Fischer, 2001; Cain and Damman, 1997) and as showed here, by the microbial community. In our case, plants inoculated with 'hedgerow' soil filtrates produced a greater number of runners than did plants treated with the inocula from forest understory. In general, plants growing in hedgerows experience high levels of inter-specific competition due to a higher plant coverage and diversity (Fahey and Puettmann, 2008). Since inter-specific plant competition in hedgerow sites is high, investment in seed production may be an unsustainable growth strategy, because the percentage of seedlings established and surviving will depend on the direct competition with other members of the hedgerow plant community. In contrast, if plants invest resources in runner production, this would be expected to increase the chances of establishment in suitable microhabitat and greater spatial competing abilities, due to the retention of physical attachment to stolons situated in already occupied, favorable habitats (Roiloa and Retuerto, 2006).

**Table 2**
Abiotic parameters at harvest of soil collected from pots inoculated with unsterilized soil inocula.

| Type | $NO_3$ (mg/kg) | $NH_4$ (mg/kg) | Org. Matter (%) | pH |
|---|---|---|---|---|
| Forest understory | 30.02 ± 6.13 | 59.17 ± 10.72 | 25.16 ± 0.71 | 6.48 ± 0.04 |
| Hedgerow | 29.09 ± 6.96 | 60.60 ± 4.81 | 25.41 ± 2.40 | 6.53 ± 0.21 |

By using an artificial mixture of soil, we avoided possible bias in plant responses due to differences in abiotic soil attributes. Consequently, any differences in plant response strategies can be attributed solely to the effect of the plant–soil microbial community. Several other studies have addressed the effect of biotic interactions on plant architecture (namely number of stolons and length), but these studies pointed to differential responses caused by the infection by plant pathogens (e.g., smut fungus and root-feeding nematodes) (Pan and Clay, 2002; D'Hertefeldt & Van der Putten, 1998). In our case, although we had already excluded certain players from our soil fauna (e.g., nematodes, mites, insect larvae), it remains difficult pinpointing a single element of the community as the causative agent for the responses observed. However, in order to have some insight into the mechanisms responsible, we analyzed root colonization by mycorrhizal fungi.

Mycorrhizal colonization is an important factor that affects both clonal growth and flowering traits (Perner et al., 2007; Wolfe et al., 2005; Streitwolf-Engel et al., 2001). Inoculation with either understory or hedgerow soil extracts resulted in similar percentages of colonization. Morphological characterization of fungal structures did not reveal any difference between the plants (in both environments fungal structures corresponded with the Glomeraceae). However, we can explain the observed differences in different ways: (i) the changes in plant architecture result from different mycorrhizal communities *in planta* in the two contrasting sites or (ii) for the same AMF community *in planta*, the bacterial community associated with mycorrhiza may be responsible for the differences in the outcome of the plant–mycorrhiza interaction (Courty et al., 2010; de Boer et al., 2005; Barea et al., 2002; Azcón, 1989). Nevertheless is important to highlight that our set-up might have biased the outcome of the plant–mycorrhiza interaction. The methods used retain mainly mycorrhizal spores smaller than 100 μm. This means that larger arbuscular mycorrhizal spores were absent. In environment such as forest soils there are also species of AM fungi with spore diameters of 100–200 μm, and these may form infective units so the results here reported cannot be attributed to the complete mycorrhizal community from the two habitats. Nevertheless, our results indicate that at least a significant fraction of the soil community can cause extreme changes in plant responses putatively beneficial within each environment.

While this study cannot elucidate the exact mechanism underlying the changes in plant growth strategies in response to the microbial community, we demonstrate that soil biota may drive aboveground plastic, and putatively adaptive strategies for plant growth, which are fundamental to understanding plant inter-specific interactions. Nevertheless, in order to fully understand the significance, further studies using other plant species and different groups of the soil community should be performed in similar and unrelated natural systems.

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