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Original article

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

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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

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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://