Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence

Giuliano Bonanomi · Max Rietkerk · Stefan C. Dekker · Stefano Mazzoleni

Abstract Positive plant-soil feedback by "ecosystem engineers" is an important driver for the structuring and organization of resource-limited ecosystems. Although ample evidence demonstrates that plant-soil feedbacks can range from positive to strongly negative, their co-occurrence in plant communities have not yet been investigated. We test the hypothesis that the plant-soil feedback generated by the nitrogen-fixer shrub *Medicago marina* during primary succession in a sand dune community has a positive effect on the coexisting grass *Lophochloa pubescens* and a negative effect on the shrub species itself. We conducted field measurements and laboratory bioassays to evaluate (1) the effects of islands of fertility on the recruitment and growth of its ecosystem engineer and on the performance of a coexisting species and (2) the mechanisms involved that can explain the opposite effects of islands of fertility on coexisting species. Islands of fertility were present under *Medicago* crowns evidenced by higher available nitrogen, extractable phosphorus and potassium, organic matter, microbial activity, water holding capacity, soil humidity, and lower salt concentrations. The effects of these islands of fertility were clearly species-specific, with a facilitative impact on *Lophochloa* and a negative effect on *Medicago* recruitment. *Lophochloa* was denser and produced more biomass when rooted inside as compared to outside the crown area of the shrub. Contrarily, the number of seedlings of *Medicago* was lower inside, despite the higher seed abundance, and higher outside the crown area of adult shrubs as compared to predictions based on random distribution, thus showing a Janzen-Connell distribution. Laboratory experiments demonstrate the occurrence of *Medicago* negative plant-soil feedback, and that the auto-toxicity of the aboveground senescent plant material is a potentially important underlying mechanism explaining this negative feedback and the resulting Janzen-Connell distribution in the field.

Keywords Auto-toxicity · Competition · Ecosystem engineers · Facilitation · *Medicago marina* · Janzen-Connell distribution · Plant-soil feedback

G. Bonanomi (✉) · S. Mazzoleni
Dipartimento di Arboricoltura, Botanica e Patologia
Vegetale, Università degli Studi di Napoli Federico II,
via Università 100, Portici 80055, NA, Italy
e-mail: giulianobonanomi@hotmail.com

M. Rietkerk · S. C. Dekker
Department of Environmental Sciences, Utrecht
University, P.O. Box 80115, Utrecht 3508 TC,
The Netherlands

Introduction

Positive plant-soil feedback by "ecosystem engineers" is an important driver for the structuring and organization of resource-limited ecosystems, such as

arid grasslands and nutrient-limited savannas (Jones et al. 1994; Pugnaire et al. 1996; Rietkerk et al. 2004). Positive plant-soil feedback is defined as the positive condition for the establishment, growth, and reproduction of plants induced by the plants themselves. For instance, trees and shrubs with extensive root systems track nutrients from the surroundings, leading to local nutrient recycling and organic matter accumulation (Belsky 1994; Schlesinger et al. 1996). The resulting resource distribution, called "islands of fertility" (Schlesinger et al. 1990), positively affects species performance (Holmgren et al. 1997; Tirado and Pugnaire 2003; Rietkerk et al. 2004). In other words, facilitation by ecosystem engineers is produced by an increase of soil water and nutrient contents. However, most empirical studies on such facilitation demonstrate positive effects on other species (Connell and Slatyer 1977; Callaway 1995), and only few cases (Callaway 1995; Wied and Galen 1997) provide evidence for direct positive effects on the offspring of the species itself.

In general, plant-soil feedback can range from positive to strongly negative (Bever 2003). Negative plant-soil feedback is defined as the negative condition for the establishment, growth, and reproduction of plants induced in the soil by the plants themselves. This has been reported for coastal sand dunes during primary succession (Van der Putten et al. 1993), secondary succession (Brown and Gange 1992; De Deyn et al. 2003), temperate grasslands (Bever 1994; Klironomos 2002; Bonanomi et al. 2005b), and temperate (Streng et al. 1989; Packer and Clay 2000) and tropical forests (Webb et al. 1967; Wills et al. 1997). Mechanisms proposed explaining this negative feedback include soil nutrient depletion (Berendse 1994; Ehrenfeld et al. 2005), the build-up of soil-borne pathogen populations (De Rooij-van Der Goes 1995; Packer and Clay 2000), the changing community composition of soil microbial organisms (Bever 1994), and the release of allelopathic compounds during organic matter decomposition causing phytotoxicity (Singh et al. 1999; Armstrong and Armstrong 2001; Bonanomi et al. 2006a). Negative feedback can affect species distribution depending on life form and propagation patterns. For instance, perennial clonal plants can move away by vegetative growth (Van der Putten 2003), while trees and shrubs can escape from "home" soil (sensu Bever 1994) via seed dispersal, producing a Janzen-Connell

distribution of seedling emergence (Packer and Clay 2000). Negative plant-soil feedback has been demonstrated to be strongly species-specific mainly affecting individuals of the same species (Oremus and Otten 1981; Van der Putten et al. 1993; Bever 1994; Singh et al. 1999; Klironomos 2002; Bonanomi and Mazzoleni 2005), as opposed to positive plant-soil feedback.

The evidence of occurrence of positive and negative plant-soil feedback is now widespread, but their co-occurrence has not been studied in combination so far. Furthermore, model studies showed how these processes can affect the spatial organization and the species diversity of plant communities (Bever 2003; Rietkerk et al. 2004; Bonanomi et al. 2005a). How negative and positive feedback co-occur on different scale and time and how this affects spatial organisation of plant community is an important area for further research (e.g. Rietkerk et al. 2004; Kéfi et al. 2007). In this study, we test the hypothesis that the plant-soil feedback generated by a nitrogen-fixer shrub species (the ecosystem engineer) during primary succession in a sand dune community has a positive effect on a coexisting grass species and a negative effect on the shrub species itself. We conducted field measurements and laboratory bioassays to evaluate (1) the effects of islands of fertility on the seedling recruitment of its ecosystem engineer and on the performance of a coexisting species and (2) the mechanisms, including intraspecific and interspecific competition, that can explain the opposite effects of islands of fertility on coexisting species.

Materials and methods

Study site description

The experimental site is a flat natural area 30 m from the shoreline of the Adriatic Sea (Central Italy, Fano 43°52′00″ N—12°58′58″ E), not influenced by the tidal level. The soil consists of gravel and sand with a very low organic matter content. The plant community is at an early stage of colonization where *Medicago marina* L., a perennial nitrogen-fixer shrub, is the dominant species with a total cover between 20 and 30%. This species generates loose canopies with an average diameter of 100 cm and an average height

of 11 cm. The surrounding soil is covered by annual species during winter and spring and is almost bare in summer. The most common annual grass is *Lophochloa pubescens* Lam., while *Agropyron junceum* L., *Eryngium maritimum* L., *Echinofora spinosa* L., *Inula crithmoides* L., *Ammophila littoralis* Beauv. and the exotic *Cenchrus incertus* Curt. are sparse (species are indicated hereafter with genera name). *Medicago* seeds germinate and seedlings establish both in autumn and early spring, whereas the *Lophochloa* annual cycle starts in spring lasting until early-middle summer.

The climate is Mediterranean, with a mean annual rainfall of 754 mm and a moderate dry summer season. Mean monthly temperatures are between 23.6°C (July) and 3.8°C (January) (averages of 50 years observation; Fano meteorological station, 5 km from study site).

Field measurements

Field measurements were carried out to: (1) quantify the effects of *Medicago* on soil characteristics and distribution of resources, (2) assess the plant-soil feedback on the seedling recruitment of the ecosystem engineer, and (3) measure the performance of the coexisting grass *Lophochloa*.

Quantification of the fertility island

Although *Medicago* crown cover was clearly discontinuous, previous plant excavation showed that root distribution was continuous, meaning that no bare soil was found without the presence of *Medicago* roots. Similar patterns of root distribution has been observed by Brisson and Reynolds (1994). Therefore, we defined three different areas of potential influence of *Medicago* shrubs: the canopy crown area (IN—influenced by litter and roots), the area immediately adjacent to the crown to evaluate if the *Medicago* canopy affected the soil (OUT$_{CLOSE}$—first 30 cm from the nearest shrub), and the area located far from shrubs (OUT$_{FAR}$—>30 cm from the nearest shrub).

During the growing season (May 2006), soil was sampled in the top 10 cm in the three areas inside and outside 10 randomly selected *Medicago* plants (crown diameter > 100 cm). This depth was chosen because the roots of most *Medicago* seedlings and adult *Lophocloa* plants were present there. Soil was sieved (mesh size 2 mm) and analyzed for pH (water), salinity, total N, ammonium, nitrate, phosphorus (Olsen method), extractable potassium (extraction in BaCl$_2$), organic matter content, and maximal water holding capacity. Microbial activity was assessed with the Fluorescein Diacetate method (FDA) that measures the amount of enzymatic activity (protease, lipase, non-specific esterase) related to organic matter decomposition (see Workneh et al. 1993 for method details). We monitored soil water content (dry weight after 72 h at +80°C) in the field for 13 days during a dry period after a rain event (10/08/2001), and compared IN and OUT$_{FAR}$ areas of 16 randomly selected *Medicago* crowns area. The amount of *Medicago* litter above and within the top soil (10 cm), was measured in 10 × 10 cm square frames randomly located in the three sampling areas (IN, OUT$_{CLOSE}$ and OUT$_{FAR}$) in 10 randomly sampled *Medicago* plants. After soil sieving (2 mm diameter), *Medicago* organic debris were dried (+80°C) and weighted.

Photosynthetic active radiation (PAR 400–700 nm) was measured (instrument ADC-L2A light sensor) at ground level in 10 large randomly selected *Medicago* individuals (crown diameter > 100 cm). Measures were done in the IN, OUT$_{CLOSE}$, and OUT$_{FAR}$ areas under bright, sunny conditions around mid-day in May 2006.

Medicago and *Lophochloa* distribution

The cover of *medicago* plants was calculated by summing all individual canopy areas (based on an average of 4 different canopy diameters). For 3 years (2001, 2003, and 2004), during the peak of seedling growth in May, the number and distance of *Medicago* seedlings to the nearest *Medicago* crown area was measured within 45 randomly sampled squared meters (plot 1 × 1 m^2) for an area of approximately 0.1 ha. The recorded distributions of *Medicago* seedlings in relation to cover type (IN and OUT) were compared with the theoretical distributions using χ^2-tests. The theoretical distributions were obtained by assigning a number of *Medicago* seedlings proportional to each class of soil areas type.

To quantify *Medicago* seed dispersal, soil, and litter were sieved (0.5 mm sieve size) and the

numbers of legumes and seeds were counted at the end of the dispersal season (November 2001). For 12 large randomly selected *Medicago* individuals (crown diameter > 100 cm), soil was sampled from the top 5 cm including undecomposed litter (size of each sample 100 cm^2) for 3 positions: IN, between 0 and 14 cm, and between 45 and 59 cm from the nearest adult *Medicago* crown area, with three sub-replicates for each position, resulting in a total of 108 soil samples. The number of seeds (average seed size 3×1.5 mm^2) per legume ranged between 2 and 5, with an average value of 2.5.

The root systems of four *Medicago* plants (crown diameter ranging from 50 to 100 cm) were excavated by hand and mapped to describe their lateral extension and depth in the soil on x–y–z coordinate system by image analysis software (LUCIA Laboratory Imaging Ltd. version 4.51). We placed tags on the excavated root systems where the diameter was less than 2 mm. Picture of the whole root systems was taken and length of thin root (diameter < 2 mm) were measured by image analysis in concentric cross-sections for every 25 cm radius by tracing the roots. Rooting depth of these excavated 4 plants and of 40 randomly selected *Medicago* seedlings were also measured.

The density of *Lophlochloa* stems and the biomass of adult *Lophochloa* plants were measured IN and OUT$_{FAR}$ 12 randomly selected *Medicago* crown areas for 3 years (2001, 2003, and 2004) at the end of the growing season (June). The number of *Lophochloa* stems was counted within squared frames (20×20 cm^2) randomly placed in each OUT$_{FAR}$ ($n = 12$) and IN area ($n = 12$). Adult *Lophochloa* plants (total number of plants > 150 for each year) rooted IN and OUT$_{FAR}$ were randomly sampled to measure dry weight (including stem, leaf, spike, and root). In 2001, the rooting depth was also measured (total number of sampled *Lophochloa* plants is 191). Root distributions of *Lophochloa* adults and *Medicago* seedlings were compared to obtain indirect information on belowground competition.

Laboratory bioassay

The first laboratory bioassays was carried out to assess the effect of IN and OUT$_{FAR}$ soil on the growth of both *Medicago* and *Lophochloa*, while the second bioassay was done to assess the effect of senescent plant material (leaves and shoots) on the survival and growth of *Medicago* seedling.

In the first experiment, soil was sampled from 10 randomly selected *Medicago* plants in the IN and OUT$_{FAR}$ *Medicago* areas, sieved (mesh size 2 mm) and successively pooled. Seeds of the two species were sampled (total number of plants > 50) and placed on Petri dishes. Pots, 12 cm diameter and 15 cm height, were filled with 250 g of air dried soil and planted with one pre-germinated 7 days old seedling of *Lophochloa* or *Medicago*, and were placed in a growth chamber (25°C, 220 µmol m^{-2} s^{-1} photons; 16:8 ratio day–night) following a complete random design with regular rotation. Pots were wetted with distilled water every 2 days until water holding capacity was reached. After 55 growing days, shoots and roots were harvested, washed with tap water, and dry weight (80°C for 72 h) was measured. The bioassay had 16 replicates with 2 soil types and 2 species resulting in a total of 64 pots.

In the second experiment, OUT$_{FAR}$ soil was sampled from 10 randomly chosen *Medicago* plants (sieved with mesh size of 2 mm and successively pooled) to assess the effect of *Medicago* leaves and shoots on the growth and survival of *Medicago*. OUT$_{FAR}$ soil was used because it was not previously affected by the aboveground litter of the same species (soil sampling and processing methods as described above). *Medicago* senescent material (leaves and shoots) was collected, dried (72 h at 80°C), and added to the pots at four levels: resulting in 0, 1, 3, and 5% plant material content (g plant material/g soil). The 1% concentration was comparable with the amount of undecomposed *Medicago* litter present in the IN area above and in the first 2 cm of soil (see Results section). Three pre-germinated 7 days old *Medicago* seedling was placed in each pot in the growth chamber as described above. Treatments were replicated 12 times resulting in a total of 48 pots. After 27 growing days, shoots and roots were harvested and washed with tap water and dry weight (80°C for 72 h) was measured. Seedling mortality, pH, and electrical conductivity of drained pot water were measured weekly.

Results

Water content of IN soil (inside *Medicago* crowns area) was higher after a rain shower, and remained

higher during the dry out period as compared to the OUT_{FAR} (outside *Medicago* crowns area) soil at all sampling dates (Fig. 1). IN soil has higher nitrate, ammonium, total nitrogen, extractable phosphorus and potassium, organic matter, water holding capacity, soil humidity, microbial activity, and reduced salinity content as compared to OUT_{CLOSE} and OUT_{FAR} soils (Table 1). Significant light attenuation occurred inside the *Medicago* canopy, while no significant differences were recorded between the OUT_{CLOSE} and OUT_{FAR} areas (one-way ANOVA; $P < 0.01$; values of PAR was IN $= 965 \pm 165b$, $OUT_{CLOSE} = 1841 \pm 46a$, and $OUT_{FAR} = 1843 \pm 30a$ μmol photon $m^{-2} s^{-1}$). *Medicago* litter was absent from the OUT_{CLOSE} and OUT_{FAR} areas, but ranged from 287 to 141 g/m^2 with an average value of 227 ± 73 g/m^2 in the IN area. *Medicago* litter partially lies above the ground, but the largest fraction was buried in the top 2 cm of the soil (G.B. personal observation). Plant biomass and stem density of *Lophochloa* was higher for plants rooted IN as compared to OUT_{FAR} for all years (Fig. 2).

Medicago seed dispersal was significantly clumped to the seed source (one-way Anova, $F = 31$, $P < 0.001$; Fig. 3) with an average of 17.265 $seeds/m^2$ IN and 229 $seeds/m^2$ at 45–59 cm from the crown edge. In contrast to the seed distribution, *Medicago* seedlings showed a Janzen-Connell distribution, with peak abundance 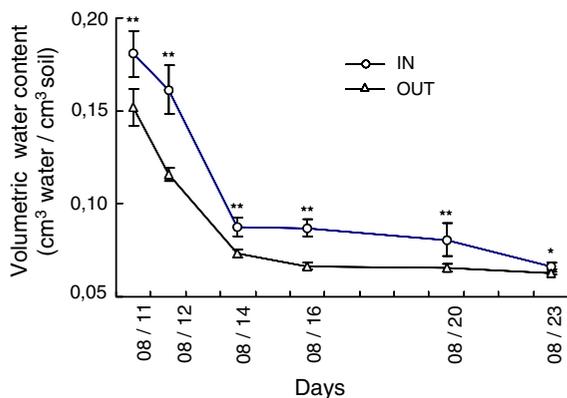at intermediate distances from the *Medicago* plant itself (Fig. 3). Observed number of seedlings was lower IN and higher OUT as compared to predictions based on random distribution for all years (Table 2). Young seedlings (<1-year-old) were relatively scarce inside *Medicago* crowns area and older seedlings were absent (Fig. 3).

The *Medicago* root systems were round-shaped with a diameter more than twice as large as compared to the crown, with highest thin root densities directly outside the crown projection (Fig. 4). After germination, *Medicago* seedlings produced a deep tap root, while *Lophochloa* adult plants showed a consistent shallow root system (Fig. 5a). Both rooting depths are logarithmic related to plant biomass (Fig. 5a). Average root length of adult *Medicago* consistently declined with depth in the soil profile, with a maximal root depth of 30 cm (Fig. 5b).

In the first bioassay experiment, *Lophochloa* growth was higher for IN soil, while *Medicago* seedling growth was higher for OUT_{FAR} soil (Fig. 6). In the second experiment, the addition of *Medicago* aboveground plant material resulted in a reduction of *Medicago* seedling growth (One-way Anova, $F = 18.7$, $P < 0.001$; Fig. 7a) and increase of mortality (One-way Anova, $F = 16$, $P < 0.001$; Fig. 7b), both depending on concentration. At higher levels of plant material addition (5% dry weight), all seedlings were wilted and showed brown roots after a few days and all died within 7 days. Plant material addition did not affect pH of the drained water and showed a weak electrical conductivity increase (<2,000 μS/cm).

Discussion

The plant-soil feedback produced by *Medicago* shrub crowns were species-specific. We found a clear positive effect on biomass and density of the coexisting grass *Lophochloa* (Fig. 2), and negative effect on *Medicago* seedling recruitment (Fig. 3). The fact that *Medicago* seed availability was much higher inside the crown area, while seedlings showed a Janzen-Connell distribution (Fig. 3) indicates a strong inhibition of both seed germination and seedling emergence (Nathan and Casagrandi 2004). The Janzen-Connell distribution of seedlings here is particularly surprising because of the presence of islands of fertility inside the crown area (Table 1).

Fig. 1 Soil moisture dynamics for 13 days after a rain event (10 August 2001) in soil inside and outside adult *Medicago marina* crown areas. Asterisk indicate significant differences within each sampling date (t-test; *$P < 0.05$; **$P < 0.01$), bars indicate ±1SE

Table 1 Soil parameters sampled in the top 10 cm under the *Medicago* canopy (IN) and outside in the area immediately adjacent to the crown (OUT$_{CLOSE}$—first 30 cm from the nearest shrub), and the area located far from shrubs (OUT$_{FAR}$—>30 cm from the nearest shrub)

Soil parameter	OUT$_{FAR}$	OUT$_{CLOSE}$	IN
pH	8.21 ± 0.07a	8.28 ± 0.09a	8.25 ± 0.08a
Salinity (μS/cm)	645 ± 53a	676 ± 65a	501 ± 25b
NO$_3$–N (mg/kg)	90.0 ± 8.1b	86.5 ± 7.6b	134.3 ± 10.6a
NH$_4$–N (mg/kg)	19.5 ± 3.17b	18.2 ± 1.75b	40.2 ± 5.66a
Total N (g/kg)	0.16 ± 0.02b	0.23 ± 0.04b	1.42 ± 0.21a
P$_2$O$_5$ (mg/kg)	6.3 ± 0.44b	6.5 ± 0.87b	39.8 ± 2.59a
K$_2$O (mg/kg)	42.1 ± 2.42b	43.1 ± 3.54b	80.5 ± 5.16a
Organic carbon (g/kg)	0.33 ± 0.14b	0.34 ± 0.15b	12.03 ± 1.81a
Soil water capacity (cm^3/cm^3)	0.15 ± 0.02b	0.16 ± 0.01b	0.23 ± 0.02a
Hydrolyzed FDA (μg g^{-1} min^{-1})	0.45 ± 0.02b	0.48 ± 0.03b	2.55 ± 0.17a

Data are averages (±1SE) of 10 replicates for all parameter. Different letters indicate significant differences (Duncan test; $P < 0.05$)
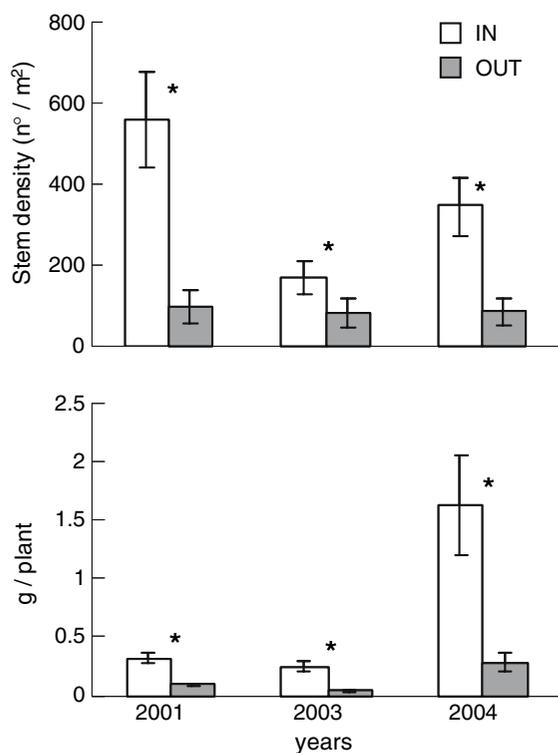

Fig. 2 Stem density (above) and plant biomass (below) of *Lophochloa pubescens* rooted inside and outside *Medicago marina* crown areas in three years. Asterisks indicate significant difference (*t*-test $P < 0.01$; $n = 12$; bars indicate ±1SE), total number of sampled individuals for measures of plant biomass was 364 in 2001, 291 in 2003 and 162 in 2004

Note, for example, that phosphorus is 6 times higher inside the crown area; a legume-like *Medicago* would normally thrive at this level.
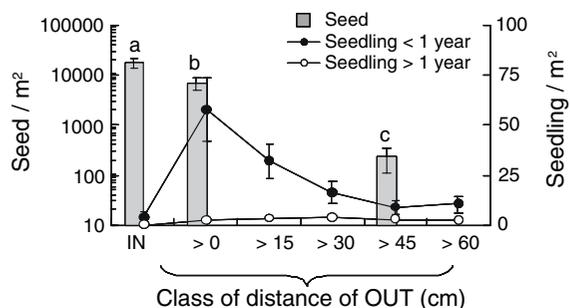

Fig. 3 Comparison between *Medicago marina* seed dispersal, <1 year seedlings and >1 year inside (IN) and outside (OUT) *Medicago marina* crowns area for 5 distances. Seedling data are averages from 2001, 2003, and 2004, seed data refer only to 2001. Different letters indicate significant differences (One-way ANOVA; Duncan test, $P < 0.05$) and bars indicate ±1SE. Data from seedling >1 year are: IN = 0; OUT$_{0–15}$ = 2; OUT$_{16–30}$ = 3.3; OUT$_{31–45}$ = 3.9; OUT$_{46–60}$ = 3; and OUT$_{>60}$ = 2.1/m^2)

Accumulating evidence indicates that ecosystem engineers drive interspecific facilitation in semi-arid environments (Callaway 1995; Pugnaire et al. 1996). In line with previous results, *Lophochloa* performed better inside as compared to outside *Medicago* crowns area, associated with the presence of islands of fertility. Facilitation through amelioration of micro-climatic conditions inside the shrub crowns is probably not involved because *Medicago* crowns are very loose, and their canopy heights are lower than *Lophochloa* plants (average 11.2 cm vs. 32.5 respectively; total number of samples = 150 for both species). This also means that aboveground

Table 2 *Medicago marina* seedling distribution (IN = inside crown area of *Medicago* plant, OUT = outside the crown area of *Medicago*), which is significantly segregated from *Medicago marina* crown in all the three sampling years

Year	*Medicago* cover (%)	Seedling (n°)				Significant level
		Predicted		Observed		
		IN	OUT	IN	OUT	
2001	29	213	523	69	667	$\chi^2 = 137.0; P < 0.001$
2003	16.6	71	355	21	405	$\chi^2 = 42.1; P < 0.001$
2004	31.5	210	455	14	651	$\chi^2 = 267.4; P < 0.001$

Data are from seedling of all age classes (<1 year and >1-year old). χ^2-tests are for the number of *Medicago* seedlings associated with a given cover type

Fig. 4 Structure of root system of two *Medicago marina* adult plants with crown diameters of 50 cm (left) and 100 cm (right). Full root systems are shown above, while only thin roots (diameter < 2 mm) are shown in the middle. Crown edges are represented as circles. The line graph (below) shows the density of thin roots (average ±1SE; total number of sampled plants = 4) in relation to distance from stem (gray shading represents the area under *Medicago* canopy)
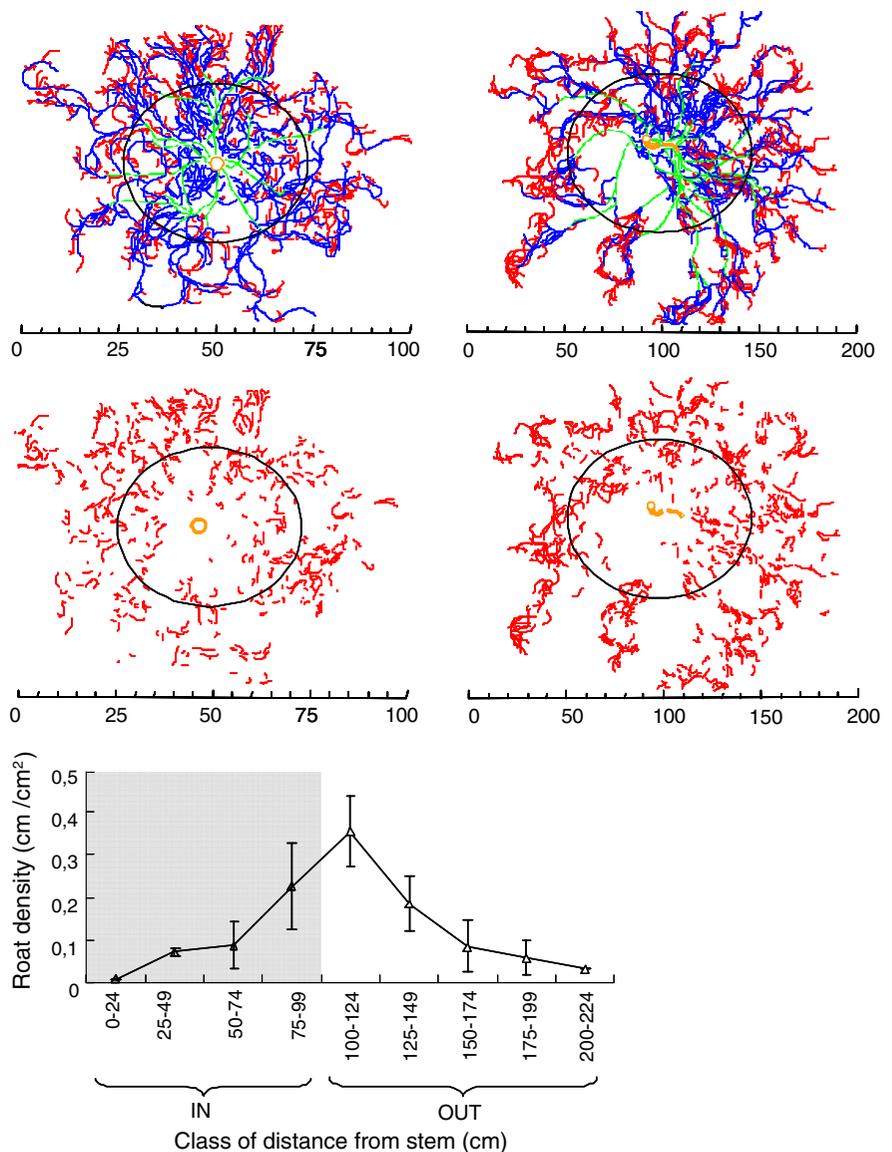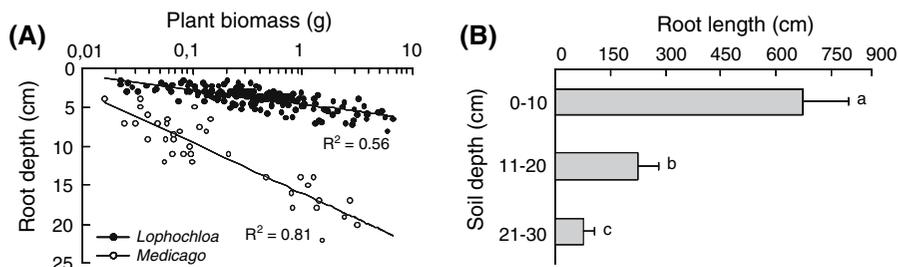
Fig. 5 (a) Relationship between plant biomass (dry weight) and maximal root depth of *Lophochloa pubescens* adult plants (total number of sampled plants = 191) and tap roots of *Medicago marina* seedlings (total number of sampled plants = 40). Lines refer to logarithmic relationships, note logarithmic

scale of the *x*-axis, (**b**) Average total root length of *Medicago marina* adult plants (total number of sampled plants = 4) at different soil depth. Different letters indicate significant difference (One-way ANOVA; Duncan test, $P < 0.05$), bars indicate ± 1SE
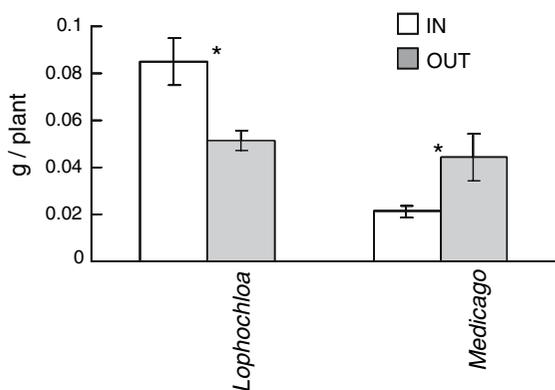

Fig. 6 Biomass of *Lophochloa pubescens* and *Medicago marina* growth on soil sampled inside and outside *Medicago* crowns area in controlled conditions. Asterisk refer to significant difference (*t*-test $P < 0.01$), bars indicate ± 1SE
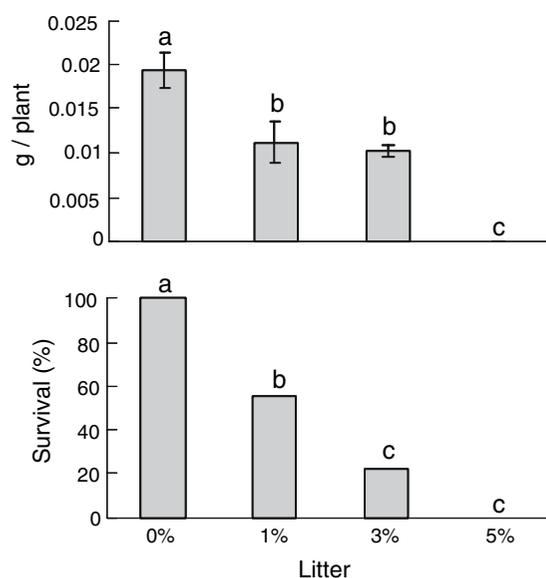

Fig. 7 Biomass (above) and mortality (below) of *Medicago marina* seedling growth on soil sampled outside *Medicago* crowns area and addition of three levels of *Medicago* senescent aboveground plant material in controlled conditions. Different letters indicate significant difference (One-way ANOVA on transformed data; Duncan test, $P < 0.05$), bars indicate ± 1SE

competitive effects for light on *Lophochloa* plants are unimportant. In fact, light at ground level is not a limiting resource in this plant community due to the low stature and density of the coexisting plants. Furthermore, belowground competitive effects on *Lophochloa* are almost absent, because available soil nitrogen and water levels are higher (Table 1; Fig. 2), and density of thin *Medicago* roots are lower inside as compared to outside the shrub crowns area (Fig. 4).

Our study indicates that auto-toxicity of *Medicago* plant material might be involved in the build-up of negative plant-soil feedback. Although *Medicago* senescent plant material was phytotoxic in the soil bioassay (Fig. 7), we acknowledge that the importance of plant material phytotoxicity and its dynamics in field conditions remains to be revealed. Field experiments that manipulate the litter amount and

that control nutrient availability and pathogens activity by using fungicides could be useful to test the importance of *Medicago* litter auto-toxicity. However, our laboratory experiment shows that this cannot be ruled out as a potential factor explaining the opposite effects of the *Medicago* plant-soil feedback on coexisting species. As far as we know, this is the first case relating a recruitment pattern to the release of phytotoxicity from decaying senescent plant material of conspecific adult plants in a natural

community. However, reduced recruitment of seedlings in the vicinity of parent plants as a consequence of accumulation of actively excreted allelopathic compounds has been reported (Webb et al. 1967; Perry et al. 2005). Phytotoxicity of decaying plant residues has been documented for many crop species (Patrick et al. 1963) and natural species (Jäderlund et al. 1996; Wardle at al. 1998; Bonanomi et al. 2006a). These studies showed that decaying plant residues, especially of nitrogen fixing species (Bonanomi et al. 2006a), exhibit the most severe inhibition at the early stage of decomposition. It is noteworthy that the crop species *Medicago sativa* is affected by a strong negative plant-soil feedback when the crop is planted in successive monocultures or in narrow rotation (Chung and Miller 1995). The negative effect on conspecific seedling recruitment decreased with increasing distance from adult plants (Jennings and Nelson 2002), as demonstrated by our case. Miller (1996) demonstrated for *Medicago sativa* that the pivotal mechanism underlying the negative plant-soil feedback is the auto-toxicity of the plant material, and that soil nutrient depletion and the build-up of pathogen populations could be ruled out as alternative mechanisms.

Field patterns of root distribution and seedling emergence of *Medicago* are consistent with the negative effect of soil below *Medicago* crowns due to auto-toxicity of its aboveground plant material. Root distribution of adult *Medicago* showed low root density under and high density outside its canopy, which is inversely related to the occurrence of islands of fertility (Fig. 4). This distribution has been previously reported for orchard (Schenk et al. 1999) and savanna trees (Belsky 1994), but no clear hypotheses have been proposed to explain this. We hypothesize that *Medicago* plants escape from the build-up of negative feedback in soil (Van der Putten et al. 1993) by developing peripheral fine roots outside their crown area.

Mechanisms other than *Medicago* auto-toxicity (Figs. 6 and 7) could also explain the observed distribution pattern of seedlings: light limitation under the canopy, hydraulic lift, increased competition between *Medicago* seedlings and *Lophochloa* or *Medicago* adult plants, nitrogen microbial immobilization inside the *Medicago* crown area, increased soil borne pathogens, and predation activity. We do not know whether *Medicago marina* is a shade tolerant or

not, and no data are available in the literature, but it is likely (based on its life history traits) that it is not. However, based on the data on PAR intensity at ground level we suggest that light is not a limiting factor for *Medicago* recruitment. In fact, although the *Medicago* canopy affects PAR intensity, the available light in the IN area remains at sufficient levels also for shade intolerant species. Moreover, we ruled out the hydraulic lift hypothesis because the maximal root depth is quite limited (35–40 cm) and the tap root of adult *Medicago* does not reach the water table (personal observation during excavation). Our evidence, although indirect, suggests a limited role of intraspecific and interspecific competition (Whittaker and Levin 1977). First of all, the level of available resources (water and major nutrients) was higher inside the *Medicago* crown area compared to outside, thus excluding resource competition. Moreover, intraspecific belowground competition between adult *Medicago* and seedlings is probably weaker inside the crown area as compared to outside, because of lower thin root density inside *Medicago* crowns area (Table 1; Figs. 1 and 4). Additionally, according with previous findings (Moles and Westoby 2004), we suggest that intraspecific competition among *Medicago* seedling would be of little relevance because they are too widely spaced for any competitive interaction. Interspecific belowground competition between *Lophochloa* plants and *Medicago* seedlings is probably low because their root systems are partially segregated in space (Fig. 5a). Moreover, the periods when the seeds of both species germinate and their seedlings establish only partly overlap (Fitter 1986; Callaway 1994), with *Medicago* seedlings germinating in autumn, while *Lophochloa* in early spring (G.B. personal observation).

Reduction of plant growth after addition of organic residues has been reported earlier (Van der Putten et al. 1997; Hodge et al. 1998) and is often related to net nitrogen (N) immobilization by microbial competition (Michelsen et al. 1995). However, this mechanism could be ruled out here because available N was higher inside *Medicago* crowns area (Table 1).

Previous studies that investigated the mechanisms of negative plant-soil feedback focused on soil-borne pathogens (Van Der Putten et al. 1993; Packer and Clay 2000). In our study, the soil experiment (Fig. 6) did not exclude pathogens, and therefore their role cannot be quantified as a possible mechanism in that

specific case. However, in the soil bioassay where the plant material was dried at 80°C (Fig. 7), the soil-borne pathogens were likely eliminated because they are more sensitive to high temperatures than saprophyte microbial communities (Martin 2003). In more general terms, it is well know that plant-pathogen interactions are affected by soil organic matter, and studies on crops species repeatedly found that soil amendment with phytotoxic organic materials increase the occurrences of soil-borne pathogen diseases (Hoitink and Boehm 1999; Bonanomi et al. 2006b). However, it seems unlikely that pathogens alone could explain the Janzen-Connell distribution because *Medicago* seedlings showed a peak immediately outside the canopy, when it is well know that soil-borne pathogens can move away into the soil at a rate of mm and in some case of cm/day as for *Pythium* spp. and *Rhizoctonia* spp. species, the most important pathogens causing seedling dumping off (Martin and Loper 1999). Based on this, it seems unlikely that seedlings rooted few centimetres apart from the crown find a safe site from soil-borne pathogens.

Finally, although we did not investigate the direct role of predation on the Janzen-Connell distribution (Janzen 1970), the abundant presence of seedlings, and the low *Medicago* susceptibility to herbivore activity by snails, the most common grazer in the community (personal observation), suggest a limited role of predation. The low *Medicago* susceptibility to herbivores could be related with the abundant presence of trichomes that completely cover the plant surface. The species is also rich in terpenes (Flamini et al. 2003) that are known for their anti-herbivore activity. No insects or traces of browsing were observed on *Medicago* seedlings, either when rooted in the IN or in the OUT areas (personal observation). Additionally, the study site is a small natural area (<10 ha) surrounded by beach with tourist facilities, which act as exclosures for large mammalian herbivores.

In conclusion, our study demonstrates that *Medicago* auto-toxicity is a potentially important mechanism explaining reduced recruitment of *Medicago* inside its crown area. Specifically, our study indicates that the release of phytotoxic plant materials could result in a negative plant-soil feedback under the crown area of adult conspecific plants, which may well be a potential alternative explanation for the

Janzen-Connell distribution. Finally, we showed that despite the clear positive soil modifications by *Medicago*, also negative effects arise under its canopy, being harmful to the recruitment of the same species, and acting in concert to promote coexistence with other plants. This might well be a more general phenomenon that deserves further investigation.

References

Armstrong J, Armstrong W (2001) An overview of the effects of phytotoxins on *Phragmitis australis* in relation to die-back. Aquat Bot 69:251–268

Belsky AJ (1994) Influences of trees on savanna productivity: tests of shade, nutrient and tree-grass competition. Ecology 75:922–932

Berendse F (1994) Litter decomposability—a neglected compound of plant fitness. J Ecol 82:187–190

Bever JD (1994) Feedback between plants and their soil communities in an old field community. Ecology 75:1965–1977

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bonanomi G, Mazzoleni S (2005) Soil history affects plant growth and competitive ability in herbaceous species. Community Ecol 6(1):23–28

Bonanomi G, Giannino F, Mazzoleni S (2005a) Negative plant-soil feedback and species coexistence. Oikos 111:311–321

Bonanomi G, Rietkerk M, Dekker SC, Mazzoleni S (2005b) Negative plant-soil feedback and positive species interaction in a herbaceous plant community. Plant Ecol 181:269–278

Bonanomi G, Sicurezza MG, Caporaso S, Esposito A, Mazzoleni S (2006a) Phytotoxicity dynamics of decaying plant materials. New Phytol 169:571–578

Bonanomi G, Giorgi V, Del Sorbo G, Neri D, Scala F (2006b) Olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens. Agric Ecosyst Environ 115:194–200

Brisson J, Reynolds JF (1994) The effect of neighbors on root distribution in a creosotebush (*Larrea tridentata*) population. Ecology 75:1693–1702

Brown VK, Gange AC (1992) Secondary plant succession: how is it modified by insect herbivory? Vegetation 101:3–14

De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, Verhoef HA, Bezemer TM, van der Putten WH (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–713

Callaway RM (1995) Positive interaction among plants. Biol Rev 61:306–349

Callaway RM (1994) Facilitative and interfering effects of *Arthrocnemum subterminale* on winter annuals. Ecology 75:681–686

Chung IM, Miller DA (1995) Effect of Alfalfa plant and soil extracts on germination and growth of Alfalfa. Agron J 87:762–767

Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. Am Nat 111:1119–1144

De Rooij-van Der Goes PCEM (1995) The role of plant-parasitic nematodes and soil-borne fungi in the decline of *Ammophila arenaria* L. Link. New Phytol 129:661–669

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant–soil system. Annu Rev Environ Resour 30:75–115

Fitter AH (1986) Spatial and temporal patterns of root activity in a species-rich alluvional grassland. Oecologia (Berlin) 69:594–599

Flamini G, Cioni PL, Morelli I, Ceccarini L, Andolfi L, Macchia M (2003) Composition of the essential oil of *Medicago marina* L. from the coastal dunes of Tuscany, Italy. Flavour Fragrance J 18:460–462

Hodge H, Stewart J, Robinson D, Griffiths BS, Fitter AH (1998) Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. New Phytol 139:479–494

Hoitink HAJ, Boehm MJ (1999) Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Ann Rev Phytopathol 37:427–446

Holmgren H, Scheffer RG, Huston A (1997) The interplay of facilitation and competition in plant communities. Ecology 78:1966–1975

Jäderlund A, Zackrisson O, Nilsson MC (1996) Effect of bilberry (*Vaccinium myrtillus* L.) litter on seed germination and early seedling growth of four boreal tree species. J Chem Ecol 22:973–986

Kéfi S, Rietkerk M, Alados C, Pueyo Y, Papanastasis VP, ElAich A, Ruiter PC (2007) Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature 449:213–217

Janzen DH (1970) Herbivores and the numbers of tree species in tropical forests. Am Nat 104:501–528

Jennings JA, Nelson CJ (2002) Zone of autotoxic influence around established Alfalfa plants. Agron J 94:1104–1111

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70

Martin FN (2003) Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. Ann Rev Phytopathol 41:325–350

Martin FN, Loper JE (1999) Soilborne plant diseases caused by *Pythium* spp. Ecology, epidemiology, and prospects for biological control. Crit Rev Plant Sci 18:111–181

Michelsen A, Schmidt IK, Jonasson S, Dighton J, Jones HE, Callaghan TV (1995) Inhibition of growth, and effects on nutrient uptake of artic graminoids leaf extracts—allelopathy or resource competition between plants and microbes? Oecologia 103:407–418

Miller DA (1996) Allelopathy in forage crop system. Agron J 36:854–859

Moles AT, Westoby M (2004) What do seedling die from and what are the implications for evolution of seed size? Oikos 106:193–199

Nathan R, Casagrandi R (2004) A simple mechanistic model of seed dispersal, predation and plant establishment: Janzen-Connell and beyond. J Ecol 92:733–746

Oremus PAI, Otten H (1981) Factor affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. Plant Soil 63:317–331

Packer A, Clay K (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404:278–280

Patrick ZA, Toussoun TA, Snyder WC (1963) Phytotoxicity substance in arable soils associated with decomposition of plant residues. Phytopathology 53:152–161

Perry LG, Thelen GC, Ridenour WM, Weir TL, Callaway RM, Paschke MW, Vivanco JM (2005) Dual role for an allelochemical: (±)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. J Ecol 93:1126–1135

Pugnaire FI, Haase P, Puigdefábregas J (1996) Facilitation between higher plant species in a semiarid environment. Ecology 77:1420–1426

Rietkerk M, Dekker SC, de Ruiter PC, van de Koppel J (2004) Self-organized patchiness and catastrophic shifts in ecosystems. Science 305:1926–1929

Schenk HJ, Callaway RM, Mahall BE (1999) Spatial root segregation: are plants territorial? Adv Ecol Res 28:146–180

Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrell WM, Virginia RA, Whitford WG (1990) Biological feedbacks in global desertification. Science 247:1043–1048

Schlesinger WH, Raikes JA, Hartley AE, Cross AF (1996) On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77:364–374

Singh HP, Batish RD, Kohli KR (1999) Autotoxicity: concept, organisms and ecological significance. Crit Rev Plant Sci 18:757–772

Streng RD, Glitzenstein JS, Harcombe PA (1989) Woody seedling dynamics in a east Texas floodplain in forest. Ecol Monogr 59:177–204

Tirado R, Pugnaire FI (2003) Shrub spatial aggregation and consequences for reproductive success. Oecologia 136:296–301

Van der Putten WH, Van Dijk C, Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56

Van der Putten WH (2003) Plant defense belowground and spatiotemporal processes in natural vegetation. Ecology 84:2269–2280

Van der Putten WH, Peters BAM, Van Den Berg MS (1997) Effect of litter on substrate conditions and growth of emergent macrophytes. New Phytol 135:527–537

Wardle DA, Nilsson MC, Gallet C, Zackrisson O (1998) An ecosystem-level perspective of allelopathy. Biol Rev 73:305–319

Webb LJ, Tracey JG, Haydock KP (1967) A factor toxic to seedlings of the same species associated with leaving roots of the non-gregarious subtropical rain forest tree *Grevillea robusta*. J Appl Ecol 4:13–25

Wied A, Galen C (1997) Plant parent care: conspecific nurse effects in *Frasera speciosa* and *Cirsium scopulorum*. Ecology 79:1657–1668

Whittaker RH, Levin SA (1977) The role of mosaic phenomena in natural communities. Theor Popul Biol 12:117–139

Wills C, Condit R, Foster RB, Hubbell SP (1997) Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. Proc Natl Acad Sci USA 94:1252–1257

Workneh F, Van Bruggen AHC, Drinkwater LE, Sherman C (1993) Variables associated with a reduction in corky root and *Phytophthora* root rot of tomatoes in organic compared to conventional farms. Phytopathology 83:581–589