APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2001, p. 495–498 Vol. 67, No. 2
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.2.495–498.2001

Management of Indigenous Plant-Microbe Symbioses Aids Restoration of Desertified Ecosystems

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Received 3 July 2000/Accepted 13 November 2000

Disturbance of natural plant communities is the first visible indication of a desertification process, but damage to physical, chemical, and biological soil properties is known to occur simultaneously. Such soil degradation limits reestablishment of the natural plant cover. In particular, desertification causes disturbance of plant-microbe symbioses which are a critical ecological factor in helping further plant growth in degraded ecosystems. Here we demonstrate, in two long-term experiments in a desertified Mediterranean ecosystem, that inoculation with indigenous arbuscular mycorrhizal fungi and with rhizobial nitrogen-fixing bacteria not only enhanced the establishment of key plant species but also increased soil fertility and quality. The dual symbiosis increased the soil nitrogen (N) content, organic matter, and hydrostable soil aggregates and enhanced N transfer from N-fixing to nonfixing species associated within the natural succession. We conclude that the introduction of target indigenous species of plants associated with a managed community of microbial symbionts is a successful biotechnological tool to aid the recovery of desertified ecosystems.

Desertification of terrestrial ecosystems is claiming several million hectares annually (29). It results from anthropogenic impacts which exacerbate the activity of natural agents. Disturbance of natural plant communities is the first visible symptom but is often accompanied or preceded by loss of key physicochemical and biological soil properties (soil structure, plant nutrient availability, organic matter content, and/or microbial activity (24). These properties largely determine soil quality and fertility, and thus plant establishment and productivity. Hence their degradation results in a loss of sustainability. Since soil degradation limits the potential for reestablishment of native plants (1, 29), erosion and desertification are accelerated. Desertification has a negative environmental impact, particularly in arid, semiarid, and subhumid areas of the world (13). In particular, desertification reduces the inoculum potential of mutualistic microbial symbionts that are key ecological factors in governing the cycles of major plant nutrients and hence in sustaining the vegetation cover in natural habitats. The most important symbionts are (i) mycorrhizal fungi, which enhance the ability of plants to establish and cope in stress situations (nutrient deficiency, drought, soil disturbance, etc.) (8, 23), and (ii) N-fixing rhizobia, which enable leguminous plants to flourish in the absence of adequate fixed N sources. A reduction in the potential to form these symbioses therefore hinders revegetation success (2, 14, 22). Mediterranean regions are characterized by a set of climatic conditions which include a long dry and hot summer, with scarce, erratic, but torrential rainfalls. This climate, together with anthropogenic degradative activities (overgrazing, nonregulated cultivation techniques, deforestation, etc.), is a major threat to the sustainability of Mediterranean ecosystems (16, 27). Susceptibility to desertification in Mediterranean regions is increasing worldwide (29). Desertified and desertification-threatened areas are common in the Mediterranean regions of Europe, particularly in southeastern Spain, and there are many representative areas where reclamation or rehabilitation programs are being attempted to restore sustainable ecosystems (12, 13, 18). Shrub communities, associated with other small woody plants, are characteristic of these semiarid ecosystems, with nitrogen-fixing legumes being key components of the natural succession (5, 12, 13, 18). These species are extremely important because their associated rhizobial symbioses constitute a source of N input to the ecosystem (7). Thus, reestablishing a shrubland is a key step in revegetation strategies. All the woody legumes involved also form a symbiosis with arbuscular mycorrhizal fungi (AMF) (13). The fungal mycelium which extends from the mycorrhizal roots forms a three-dimensional network which links the roots and the soil environment. It constitutes an efficient system for nutrient uptake (particularly P) and scavenging in nutrient-poor conditions. The mycelium also contributes to the formation of water-stable aggregates necessary for good soil tilth (15). In the tripartite rhizobial-AMF-legume symbiosis, there is synergism between the partners in that the scarcity of available P in desertified ecosystems limits legume establishment and N_2 fixation (7) in the absence of AM formation. Loss of microsymbiont propagules from degraded Mediterranean ecosystems can preclude either natural or artificial processes of revegetation; therefore, augmentation of the inoculum may be needed (20). In revegetation schemes, inoculation of plants with microsymbionts should not only help plant establishment (13) but also improve the physical, chemical, and biological soil properties contributing to soil quality (10). This premise has to be tested experimentally.

The main objective of this investigation was to assess the long-term benefits of inoculation with a combination of AMF and

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rhizobial strains on the establishment of key plant species in a representative desertified Mediterranean ecosystem. The experimental variables to be tested in transplanted seedlings included survival rates, N fixation, N transfer from N-fixing to nonfixing species associated in the natural succession, and improvements in soil quality in terms of N content, levels of organic matter (OM), and hydrostable soil aggregates. In addition, we determined whether these changes were accompanied by an increase in the inoculum potential of AMF, suggesting that this represented the initial steps in the restoration of a self-sustaining ecosystem.

MATERIALS AND METHODS

The ecosystem. A representative area within a desertified semiarid ecosystem in the Sierra de los Filabres, Almeria, southeastern Spain, was chosen for field studies. The existing natural vegetation was a degraded shrubland where *Anthyllis cytisoides*, a drought-tolerant legume able to form symbioses with both rhizobial and AM microsymbionts, was the dominant species (20). Small numbers of indigenous AMF spores (*Scutellospora calospora*, *Glomus coronatum*, *G. constrictum*, *Acaulospora* spp., and an undescribed hyaline species typical of semiarid soils [11]) were present in the soil, but the indigenous inoculum potential of AMF was inadequate to support an extensive revegetation program (20). Two experiments were carried out.

Experiment 1. Seeds of *A. cytisoides* were collected at random from wild plants growing in the experimental area and germinated (20). After 3 days, seedlings were transferred to 325-ml plastic containers (Troncoconic), as used in commercial forestry practice, filled with 300 g of autoclaved soil from the field site. Plants (40 per treatment) were grown under nursery conditions at ambient temperatures from 19 to 25°C, with watering every 2 days. All seedlings were inoculated with a previously selected rhizobial culture, *Rhizobium* sp. strain NR4 (from the collection of this institute [21]), and received one of three mycorrhizal treatments: (i) inoculation with an exotic AMF, *Glomus intraradices*, from the Consejo Superior de Investigaciones Científicas collection; (ii) inoculation with a mixture of indigenous AMF; or (iii) a control treatment of sterile inoculum. Inoculation was performed such that each seedling received 20 g of inoculum (21) consisting of a mixture of spores, hyphae, and mycorrhizal onion root fragments from trap cultures containing either *G. intraradices* or all five AMF taxa and representing the natural abundance and diversity in the site. The control was an autoclaved mixture of these AMF inocula.

After 6 months, in November 1994, the plants were transplanted to the chosen desertified ecosystem (20). The plants were planted 1 m apart in a randomized design in a bare area at a density similar to the natural abundance in well-covered areas in the adjacent ecosystem. The plants were left to develop under field conditions without any further management. After 12, 36, and 60 months, the plants were assessed for percentage survival. Fifteen representative plants per treatment were selected for monitoring of the key experimental variables in the adjacent rhizosphere soil, such as soil aggregation (9), nitrogen and organic matter content (9), number of AMF infective propagules (10), and height and diameter of the plant canopies. In each case, rhizosphere soil was collected by using a corer. Climatological characteristics are described elsewhere (19), but the key features were as follows: average annual rainfall, 218 mm; temperature range, 4 to 30°C; average yearly temperature, 18°C.

Experiment 2. Two-month-old seedlings of both *A. cytisoides* and *Lavandula multifida* plants were transplanted into an adjacent experimental area to that used for experiment 1. The plants were produced as above, except that only two treatments were compared (plus or minus the inoculum of indigenous AMF). The *Anthyllis* seedlings were inoculated with a rhizobial culture as before. A completely randomized block design was used for planting, consisting of two factors: planting combinations and AMF inoculation treatments. The planting combinations included (i) *Anthyllis* growing alone, (ii) *Lavandula* growing alone, and (iii) the plants growing as a mixture of the two species. In all cases, the plants were planted 20 cm apart to facilitate rhizosphere interactions. The other factor, the AMF treatment consisted of (i) plants inoculated with the mixture of indigenous AMF as prepared for experiment 1 and (ii) the noninoculated control (also prepared as in experiment 1). There were 20 plants per species/planting combination and AMF treatment. All plants received a single dressing at transplanting of the isotope ^{15}N as $(NH_4)_2SO_4$ with 10% ^{15}N atom excess, at a rate equivalent to 5 kg of N ha^{-1} to measure N fixation and N transfer from the N-fixing to the non-N-fixing plants (7). After 10 months of growth under natural conditions in the field, the plants were harvested and shoot biomass was determined after drying for 48 h at 60°C. Isotopic N composition was determined (7), and the roots were evaluated for percent colonization by AMF (21).

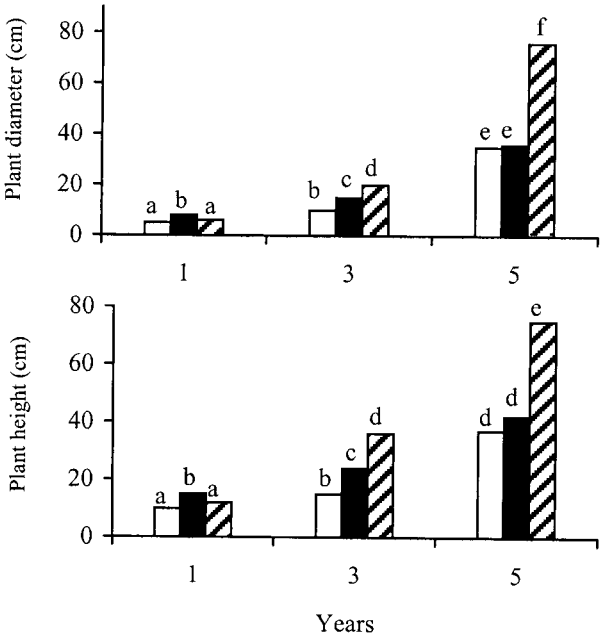

FIG. 1. Time course changes in plant growth of field-established nodulated *A. cytisoides* growing under natural conditions, either uninoculated (□) or inoculated with *G. intraradices* (■) or with native AMF (▨). For each experimental variable, mean values not sharing a letter differ significantly at $P < 0.05$ by Duncan's multiple-range test.

RESULTS

Experiment 1. The results of a 5-year trial showed significant improvements in the performance of *Anthyllis* plants inoculated with native AMF and rhizobial ecosymbionts (Fig. 1). Survival rates were higher in AMF-inoculated than in uninoculated plants (80 and 65%, respectively), but at least 25 plants in any treatment, out of the 40 transplanted, survived the dry and adverse conditions during the first year after outplanting, with no more losses afterward. In year 1, the plants inoculated with the exotic AMF *G. intraradices* were larger than those that underwent the other two treatments, but by year 3 the plants inoculated with the mixed indigenous AMF were the largest. By year 5, the plants inoculated with *G. intraradices* were not significantly larger than the plants not inoculated with AMF whereas the plants inoculated with the mixed inoculum were almost twice as large as those that underwent the other two treatments.

Inoculation with the microbial ecosymbionts resulted in an increase in the number of AMF propagules able to develop colonization units on plant roots in the soil around the *Anthyllis* plants (Fig. 2). Diversity analysis of AMF propagules in the rhizosphere of *Anthyllis* plants showed that all five key taxa were present in the rhizosphere of all plants in year 5 while spores of the introduced exotic AMF were scarce.

There were also significant improvements in years 3 and 5 in the physicochemical properties in the soil around the *Anthyllis* plants inoculated with the mixed AMF inoculum, including N content, amount of OM, and aggregation (Fig. 2).

Experiment 2. The 1-year field trial used ^{15}N isotope dilution techniques to study N fixation in *Anthyllis* and N transfer from this plant to *Lavandula*, a nonleguminous woody species commonly associated with *Anthyllis* in the natural plant suc-

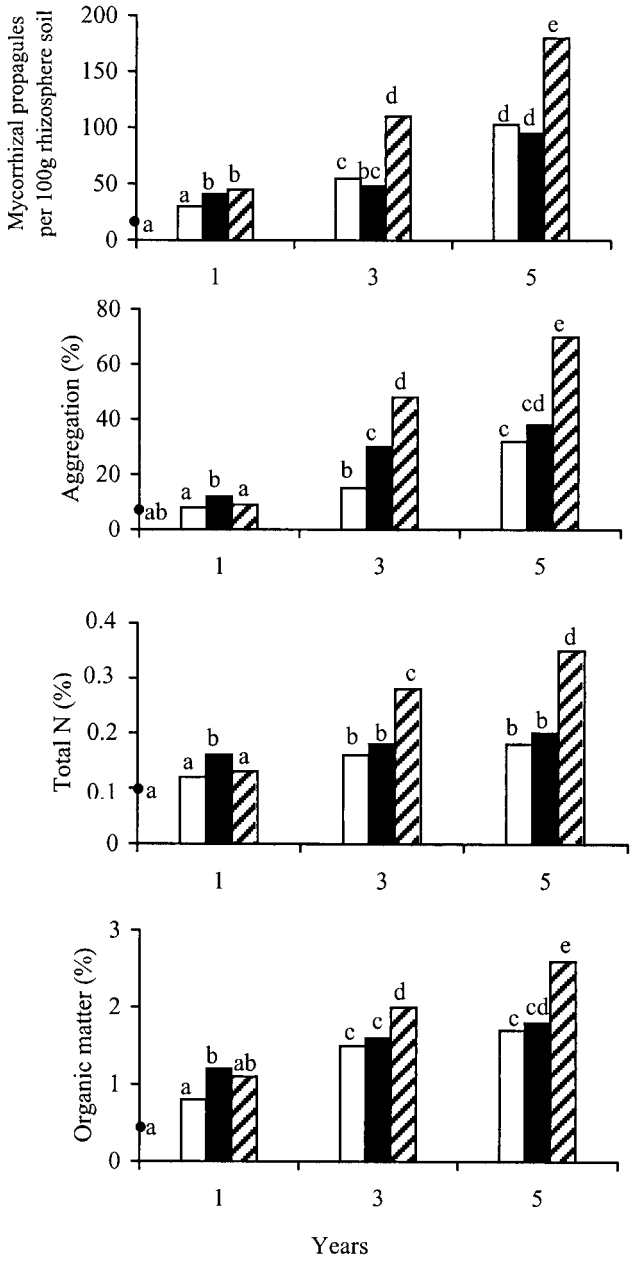

FIG. 2. Time course changes in soil traits related to soil quality in the rhizosphere of field-established nodulated plants of *A. cytisoides* growing under natural conditions, either uninoculated (□) or inoculated with *G. intraradices* (■) or with native AMF (▨). For each experimental variable, mean values not sharing a letter differ significantly at $P < 0.05$ by Duncan's multiple range test. Data points on the *y* axis represent background values in the bare soil before transplanting.

cession in the target area (5). The results (Fig. 3) showed that (i) *Lavandula* plants benefited from growing with the N-fixing legume, with regard to both biomass accumulation and N acquisition; (ii) inoculation with native AMF benefited plant growth, N fixation, and N transfer in both plants, even though indigenous fungi had colonized the roots of noninoculated plants to high levels after 10 months in the field; and (iii) inoculation of *Anthyllis* with AMF also enhanced the mycorrhizal level of uninoculated *Lavandula* plants growing nearby.

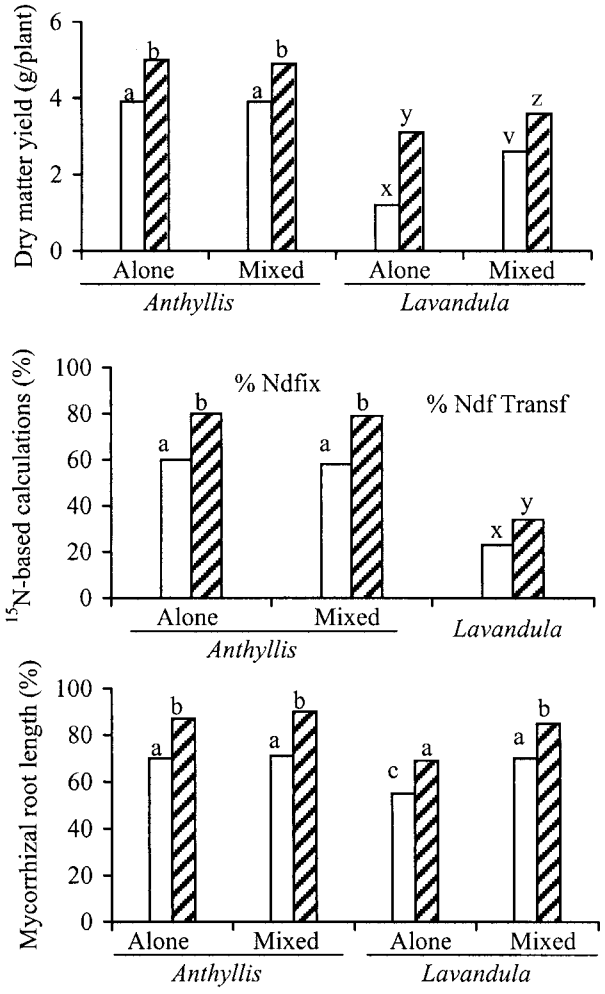

FIG. 3. Above-ground biomass production, amount of N in the legume derived from biological fixation (%NdfFix), amount of N in the nonlegume derived from transfer of the N fixed by the legume (%NdfTransf), and mycorrhizal colonization in plants of *A. cytisoides* and *L. multifida* growing for 10 months under natural conditions, either uninoculated (□) or inoculated with native AMF (▨). For each experimental variable, mean values not sharing a letter differ significantly at $P < 0.05$ by Duncan's multiple-range test.

DISCUSSION

In relation to applied environmental microbiology, the following three main points deserve discussion: (i) whether the microsymbionts, particularly native versus exotic AMF, were effective in improving outplanting performance and survival of native legumes; (ii) whether a long-term effect of AMF inoculation on physicochemical soil properties was evident; and (iii) whether AMF inoculants affect N fixation by the target legume and the subsequent N transfer to improve N nutrition for non-N-fixing vegetation.

The effectiveness of microsymbionts in improving the outplanting performance of native shrub legumes was evident. Results from experiment 1 showed that bioaugmentation of the soil with an inoculum of a mixed, native AMF inoculum increased plant productivity. This correlates with other studies which show that native AMF are important contributors to plant biodiversity and ecosystem productivity (3, 28). It is noteworthy that all five key

taxa of AMF propagules were present in the rhizosphere of all plants in year 5, while spores of the introduced exotic AMF were scarce. Thus, the inoculum had established sustainable relationships with the transplants and would maintain the inoculum potential of the ecosystem. These results demonstrate that this biotechnology (microsymbiont inoculation) can be used in revegetation strategies for desertified lands.

A long-term improvement in the physicochemical properties was evident in the soil around the *Anthyllis* plants inoculated with the mixed AMF inoculum, including increased N content and higher levels of OM and soil aggregation. An increase in the levels of both OM and N in soil stimulates plant development (12, 18, 27). The OM content increases mainly through leaf and branch fall, but it has also been related to the extent of AM colonization of the root (10). It can be assumed that the increase in N content in the rhizosphere of the legume can be accounted for by an improvement in nodulation and N fixation capacity resulting from inoculation with AMF (7). The improvement of soil aggregation contributes to the maintenance of good water infiltration rates, good tilth, and adequate aeration for plant growth, thus improving soil quality (30). The important role of the soil mycelium of AMF in the formation of water-stable soil aggregates is well documented (4, 9, 17, 26), and the involvement of glomalin, a glycoprotein produced by the external hyphae of AMF, has been demonstrated (30). Glomalin has been suggested to contribute to the hydrophobicity of soil particles and also, because its glue-like hydrophobic nature, to participate in the initiation of soil aggregates (30).

It is clear from the results of the experiment 2 that inoculation with native AMF benefited plant growth, N fixation, and N transfer. An improved N status of nonleguminous plants grown in association with legumes has previously been described for agricultural crops (6, 15), but this is the first demonstration of this phenomenon for natural plant communities in a semiarid ecosystem. The results emphasize the important role of shrub legumes as a source of AMF inoculum for the surrounding area and in improving N nutrition for non-N-fixing vegetation. They support the general conclusion that the introduction of target indigenous species of plants associated with a managed community of microbial symbionts is a successful biotechnological tool to aid the recovery of desertified ecosystems.

ACKNOWLEDGMENTS

This work was supported by the CICYT, OECD, Wain Fund, and EU REDEEM, REDMED and MYRISME Projects.

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