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A revegetation strategy based on the management of arbuscular mycorrhizae, *Rhizobium* and rhizobacterias for the reclamation of desertified Mediterranean shrubland ecosystems

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SUMMARY - A research programme is proposed for shrubland ecosystem recovery, i.e., to improve soil stabilization, and to restore a stable and diversified matorral in certain pilot-units representative of a Mediterranean desertified semi-arid ecosystem. The research approach is based on enhancing the colonization ability of woody legumes belonging to the natural succession. In this context, legumes are of special importance since they are able to take advantage of their ability to fix N_2 in symbiosis with *Rhizobium*, and to form mycorrhizae. In this symbiosis the fungal partner colonizes and links root and surrounding soil to play a critical role by improving plant rooting and establishment, helping plants to cope with stress situations such as nutrient deficiencies, drought, contamination and soil disturbance. Plant Growth Promoting Rhizobacteria will be also applied. Because soil erosion reduces the number and activity of mycorrhizal propagules the restoration of the mycorrhizal potential could be a key factor to enhance the ecosystem recovery rate. It is proposed to follow parameters related to "soil quality" (aggregate formation, organic matter, N, P levels, micronutrient equilibria, etc.), plant cover development (plant growth, nutrient acquisition, stress resistance, diversity, demography, etc.), system ability for "self-mycorrhization", etc. Current methodological approaches will be applied, such as those for rhizosphere biotechnology (molecular biology, isoenzymatic profiles, etc.), isotopic techniques (^{15}N , ^{32}P , ^{13}C), analytical methods in physicochemistry, ecophysiology, biometry, etc.

Key words: *Rhizobium*, N_2 -fixation, mycorrhiza, rhizobacteria, desertification, natural succession, shrublands.

RESUME - "Stratégie de reboisement basée sur la gestion de mycorhizes arbusculaires, rhizobiums et rhizobactéries pour la restauration d'écosystèmes arbustifs méditerranéens désertifiés". Un programme de recherche est proposé pour le rétablissement de l'écosystème arbustif, en particulier, pour améliorer la stabilisation du sol et rétablir un taillis stable et diversifié dans certaines unités pilotes représentatives de l'écosystème méditerranéen semi-aride désertique. L'approche scientifique est basée sur l'amélioration de l'aptitude à la colonisation de Légumineux arborescents appartenant à une succession naturelle. Les Légumineux sont avantagés par leur capacité de fixer l'azote (N_2) en symbiose avec *Rhizobium*, et de former des mycorhizes. Dans cette symbiose, le partenaire fongique colonise et se lie aux racines et au sol environnant pour jouer un rôle primordial, notamment en favorisant l'établissement et l'enracinement de la plante, et en permettant aux plantes de faire face à diverses situations de stress tels qu'un déficit nutritif, la sécheresse, la contamination et la modification du sol. Les rhizobactéries favorisant la croissance des plantes (PGPR) seront aussi étudiées. Parce que l'érosion du sol réduit le nombre et l'activité des propagules mycorhiziens, la restauration du potentiel mycorhizien pourrait être un facteur-clé pour l'augmentation du pourcentage de rétablissement de l'écosystème. Il est proposé de suivre les paramètres liés à "la qualité du sol" (formation d'agrégats, matière organique, niveaux de P et N, équilibre en micro-éléments), au développement du

recouvrement par les plantes (croissance des plantes, acquisition de nutriments, résistance aux stress, diversité, démographie, etc.), à la capacité du système à une auto-mycorhization etc. Seront appliquées des approches méthodologiques classiques telles que celles employées dans la biotechnologie de la rhizosphère (biologie moléculaire, profils isozymiques); marquages isotopiques (^{15}N , ^{32}P , ^{13}C), méthodes analytiques en physico-chimie, écophysiologie, biométrie etc.

Mots-clés : Rhizobium, N_2 -fixation, mycorhizes, rhizobactéries, désertification, succession naturelle, lande arbustive.

Introduction

A key premise in ecosystem preservation which has been universally accepted derives from the concept of sustainability. In this context, it is important to realize that a sustainable ecosystem can be achieved by means of the rational use of natural resources. In this way environmental quality can be maintained in general, and in particular, the structure and diversity of plant communities can be preserved (Barea and Jeffries, 1995). A sustainability-based approach is critical to propose revegetation strategies. In order to do that properly, it is critical that these are based on sustainability.

It is known that the natural equilibrium of a given ecosystem can be disturbed by changes in the activity of natural agents (climatic, geomorphic or paleo-tectonic processes, etc.). Consequently, structure, morphology and species combination, which are characteristic of the vegetation cover, can become degraded. This fact is concomitant with a generalized damage of the biological, chemical and physical status of the soil (Francis and Thornes, 1990; Morgan *et al.*, 1990). A decline in soil and plant productivity, particularly in areas subject to moisture deficiency, ultimately leads to a desert situation ("desertization"). Anthropogenic activities can cause or accelerate desertization processes, thus resulting in either a "degraded" or "desertized" ecosystem, depending upon the severity of the disturbance (Allen, 1988). Degraded ecosystems can be returned to their original conditions by applying appropriate management technologies to soil-plant systems (restoration) (Allen, 1988). In theory, desertified ecosystems are irreversible. However, suitable strategies for revegetation, based on ecological principles, can be developed to recover vegetation.

The aim of this paper is to propose a strategy, and outline the corresponding methodological approaches, for reestablishing a shrubland (matorral) cover based on the appropriate management of microbial-plant interactions. Accordingly, we will analyse first: (i) the situation regarding desertification in Mediterranean ecosystems; (ii) the ecological significance of soil microorganisms, as a key component of the sustainability in natural ecosystems; and (iii) the general types of revegetation strategies, and the related biotechnological inputs.

Desertification in Mediterranean ecosystems

The characteristics of desertification include (Herrera *et al.*, 1993): loss or disturbance of the vegetation cover, increase in soil erosion, loss of available nutrients and organic matter, loss of microbial propagules and/or diminution in microbiota

activity, thus affecting suitable nutrient cycling. These determinants act either as causes or effects thereby creating a downward spiral, the result of which is the progressive degradation of both soil fertility level and vegetation. This causes a decline in plant productivity and in the degree of soil protection against further activity from erosive agents.

Mediterranean ecosystems are subject to a set of particular climatic conditions in which scarce and irregular rainfall is a key determinant. There is often a characteristic very long dry period in the summer, usually lasting for several months. Desertification can become a serious problem where the precipitation regime is particularly erratic, and it is combined with anthropogenic pressure exerted over a long time. Desertified Mediterranean ecosystems, are very fragile and subject to progressive disturbance of the vegetation cover (López-Bermúdez and Albaladejo, 1990) and the rapid erosion of surface soils.

The microbial component in natural ecosystems

Either in natural ecosystems or in agroecosystems, sustainability is dependent on a biological balance in the soil (Barea and Jeffries, 1995), which is mainly governed by the activity of microbial communities, some of such activities can be managed as a natural resource tool (Bethlenfalvay and Linderman, 1992). Many of the soil-borne microbes are bound to the surface of soil particles or found in soil aggregates, while others interact specifically with the plant root system (Glick, 1995); actually, a large number of microorganisms are living in the soil-plant interfaces where a microcosm system, the rhizosphere, develops (Lynch, 1990; Azcón-Aguilar and Barea, 1992; Linderman, 1992).

Soil microbial dynamics largely govern ecosystem functioning (Kennedy and Smith, 1995) through a number of activities, carried out mainly by rhizosphere microbiota constituents, which are known to enhance soil and plant quality (Bethlenfalvay and SchYepp, 1994). The functions include: improvement of plant establishment, increased availability of plant nutrients, enhancement of nutrient uptake, protection against cultural and environmental stresses, improvement of soil structure, etc. (Barea and Jeffries, 1995). Certainly, plant health and productivity depend on soil quality which, in turn, is dependent on the diversity and effectiveness of its microbiota (Bethlenfalvay and SchYepp, 1994).

Certain microorganisms are particularly beneficial to plant growth and health. These can be integrated into two main groups: (i) saprophytes and (ii) mutualistic symbionts. Among other microbial types, the saprophytes include the so-called Plant Growth Promoting Rhizobacteria (PGPR). These bacteria participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment (Kloepper, 1992; Glick, 1995). Mycorrhizal fungi and N₂-fixing bacteria are among the most important members within the influential group of mutualistic symbionts. The mycorrhizal fungi, upon the biotrophic root colonization, develop an external mycelium which is in fact a bridge connecting the root with the surrounding soil microhabitats. Symbiotic N₂-fixing associations, involving *Rhizobium*, *Frankia* and cyanobacteria, are also relevant to sustainable systems. (Olivares *et al.*, 1988; Danso *et al.*, 1992)

Mycorrhizal symbiosis

This fungal-plant (root) association plays a key role in nutrient cycling in ecosystems. Additionally, the external mycorrhizal mycelium, in cooperation with other soil organisms, forms water-stable aggregates necessary for good soil tilth. Mycorrhizae improve plant health through increased protection against biotic and abiotic stresses (Bethlenfalvay and Linderman, 1992).

Mycorrhizal associations can be found in nearly all ecological situations, and most plant species are able to form this symbiosis naturally, the arbuscular mycorrhizae (AM) being the most common type involved both in the normal cropping systems and in natural ecosystems (Harley and Smith, 1983). The responsible symbiotic fungi (AM fungi) belong to the order *Glomales* in the *Zygomycetes* (Rosendahl *et al.*, 1994).

The role of AM in determining plant community structure and in successional processes (Read, 1993; Francis and Read, 1994), in crop production (Barea *et al.*, 1993), and in revegetation, (Jasper 1994) has been recently reviewed. It is well known that the symbiosis enhances the ability of the plant to establish and cope with stress situations (nutrient deficiency, drought, trace element imbalance, soil disturbance), typical in desertification (Barea *et al.*, 1990).

Revegetation strategies and related biotechnological inputs

As stated before, although a desertified ecosystem is theoretically irreversible, suitable strategies for revegetation, can be carried out to recover vegetation. The use of preexisting species could return the desertified ecosystem to a climax-like ecological functioning (reclamation) (Allen, 1988). Conversely, another management strategy is that of using exotic species, or local species from different ecosystems. New stable and sustainable ecosystems are then engineered, but with a different land use (rehabilitation) (Allen, 1988).

The establishment of a suitable plant cover is known to improve the chemical, physical, and biological properties of the soil (Skujins and Allen, 1986; Francis and Thornes, 1990; Morgan *et al.*, 1990). However, the scarcity of microbial propagules in the eroded soil (Jasper, 1994; Barea and Jeffries, 1995) could become a handicap to plant establishment because the formation of a dynamic rhizosphere is critical, particularly in low nutrient ecosystems.

The indigenous flora of the Mediterranean region is usually dominated by characteristic semi-arid shrub communities mainly formed by small woody plants. This particular type of plant cover must be considered in a revegetation programme (Francis and Thornes, 1990).

Particularly, woody legumes are useful for revegetation of water deficient ecosystems having a low availability of N, P and other nutrients (Barea *et al.*, 1992a, b), because of their ability to develop symbiotic associations with both rhizobial bacteria and mycorrhizal fungi. *Rhizobium* or *Bradyrhizobium* spp. have been isolated

from root nodules of different woody legumes but knowledge is scarce concerning the selection of microsymbionts to realize the full potential of a given woody legume/rhizobia combination, to maximise biological N₂ fixation. The scarcity of available P and the imbalance of trace elements in desertified ecosystem actually limit legume establishment and N₂ fixation (Barea *et al.*, 1992a, b). Thus, mycorrhizas have been found to benefit legume performance. Moreover woody legumes usually have a considerable degree of dependency on mycorrhiza to thrive in stressed situations (Barea and Honrubia, 1993; Herrera *et al.*, 1993). Arbuscular mycorrhizae, by far the most widespread in nature, are the commonest in nodulated, N₂-fixing legumes (Barea *et al.*, 1992a,b).

Therefore, interacting mycorrhizal and rhizobial symbioses seem to be, therefore, important to enhance revegetation. However, as soil erosion tends to reduce mycorrhizal propagules (Jasper, 1994), it could be critical to reintroduce them to improve the recovery rate of disturbed ecosystems. A selection of mycorrhizal fungi for symbiotic efficiency/physiological compatibility with the test legume ("functional compatibility" (Smith and Gianinazzi-Pearson, 1988) must be first carried out.

Herrera *et al.*, (1993) have reported the results of a four-year field revegetation trial carried out in a semi-arid desertified ecosystem in south-eastern Spain. These desertified ecosystems are appropriate for testing rehabilitation programmes (Allen, 1988; Francis and Thornes, 1990; Morgan *et al.*, 1990). Herrera *et al.* (1993) assessed the significance and effectiveness of plant-microbe symbioses as a component of a revegetation strategy in which a number of woody species, common in revegetation programmes in Mediterranean regions, were used. These included two native shrubs (*Anthyllis cytisoides* and *Spartium junceum*) and four exotic tree legumes (*Robinia pseudoacacia*, *Medicago arborea*, *Acacia caven* and *Prosopis chilensis*). Plant species and microsymbionts were screened for appropriate combinations, and a simple procedure to produce plantlets with an optimized mycorrhizal and nodulated status was developed. During a four-year period after outplanting, the results showed that: (i) only the native shrub legumes were able to get established under the local environmental conditions; (ii) inoculation with rhizobia and AM fungi improved plant survival, and biomass development. Since the two native shrubs are found in the natural plant community they could be appropriate for revegetation of these desertified areas.

A proposal for reestablishing a shrubland cover based on managing microbial-plant interactions

According to the statements above mentioned a reclamation strategy was therefore proposed for general revegetation programmed, using *Anthyllis cytisoides*, a particularly drought-tolerant species as a key plant. *Anthyllis cytisoides* is known to be mycorrhizally responsive at low P levels (López-Sánchez *et al.*, 1992 and unpublished observations). The proposed technique involves the artificial acceleration of natural revegetation, and could be accomplished by replanting randomly spaced groups of shrubs, according to the natural pattern and structure of the undisturbed ecosystem (Francis and Thornes, 1990; Morgan *et al.*, 1990; Herrera *et al.*, 1993).

To develop such a strategy a particular site was chosen to be used as a pilot study zone representative of a desertified Mediterranean ecosystem. It includes a natural

community of woody legume plants in which the shrub legume *Anthyllis cytisoides* dominates. For this reason, this species has been chosen as a test plant to investigate whether mycorrhizal technology can be used to accelerate the natural process of revegetation of the pilot site.

The area chosen for such a general programme of revegetation is situated in a sedimentary basin about 600-800 m high, located in the Sierra de Filabres, Almería (Southern Spain). The mean annual precipitation is 230 mm. The soil is an Eutric Regosol. The experimental plots are established on a formerly cultivated area, abandoned fifty years ago. The natural vegetation of this area now comprises three main shrub species: *Anthyllis cytisoides*, *Stipa tenacissima* and *Retama sphaerocarpa*, and a variety of small graminaceous species, such as *Stipa capensis*. The most important of these shrubs is in fact *Anthyllis cytisoides*. This species accounted for more than 60% of the shrub vegetation of the area. Several other shrub species also grew in this area, such as *Artemisia herba alba* and *Thimelaea hirsuta*.

The main objectives of the programme are outlined in Fig. 1, while the methodological approaches are summarized in Figs 2, 3 and 4.

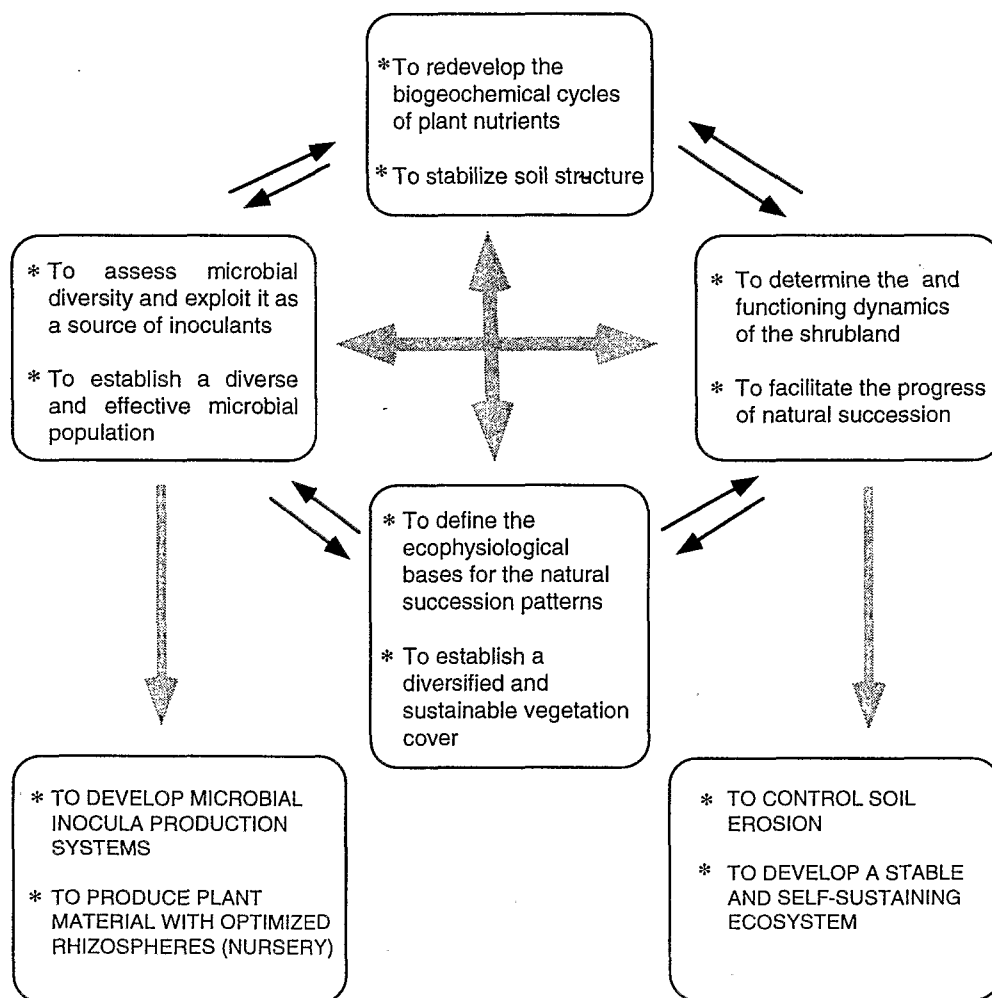


Fig. 1. Microorganisms in revegetation programmes for desertification control. Main objectives.

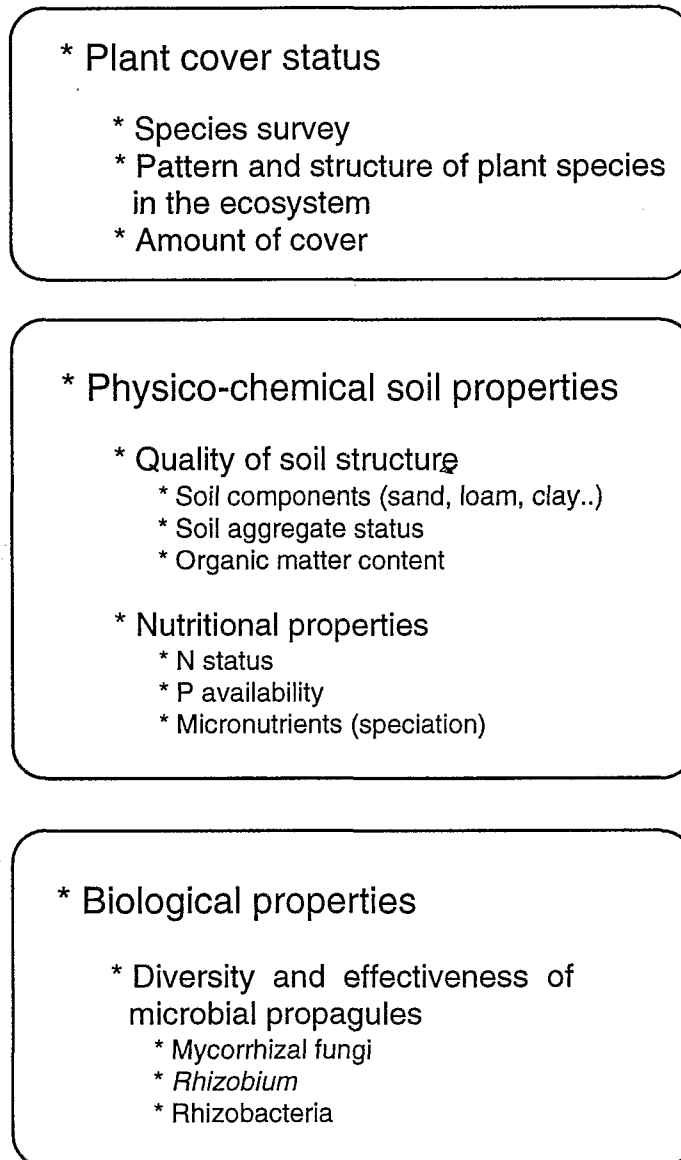


Fig. 2. Microorganisms in revegetation programmes for desertification control. Evaluation of the target ecosystem degradation.

From the biotechnological/practical point of view, it must be stated that, while rhizobial and rhizobacterial inocula can be easily formulated, the mass production of mycorrhizal inocula has well-known difficulties (Thompson, 1994). Nevertheless, mycorrhizal biotechnology can be integrated into nursery and revegetation management, which is mainly based on the use of transplants of vegetatively-produced plant material. It appears that appropriate microsymbionts management can help legumes to promote the stabilization of a self-sustaining ecosystem (Barea and Jeffries, 1995). The already mycorrhizal shrubs, acting as a "fertile islands" (Skujins and Allen, 1986), could serve as sources of inoculum for the surrounding area and to improve N nutrition for the non N-fixing vegetation in these semi-arid ecosystems.

Current methodological approaches are applied such as those for rhizosphere biotechnology (molecular biology, isoenzymatic profiles, etc.); isotopic techniques (^{15}N , ^{32}P , ^{13}C), analytical methods in physico-chemistry, ecophysiology, biometry etc. These will be used to evaluate either plant or soil parameters, critical to ascertain the level of soil quality, which is a decisive factor for plant development, and also the level of plant performance, which is a decisive factor for improving soil quality. Our thesis is that appropriate management of soil microorganisms can improve both plant performance and soil quality.

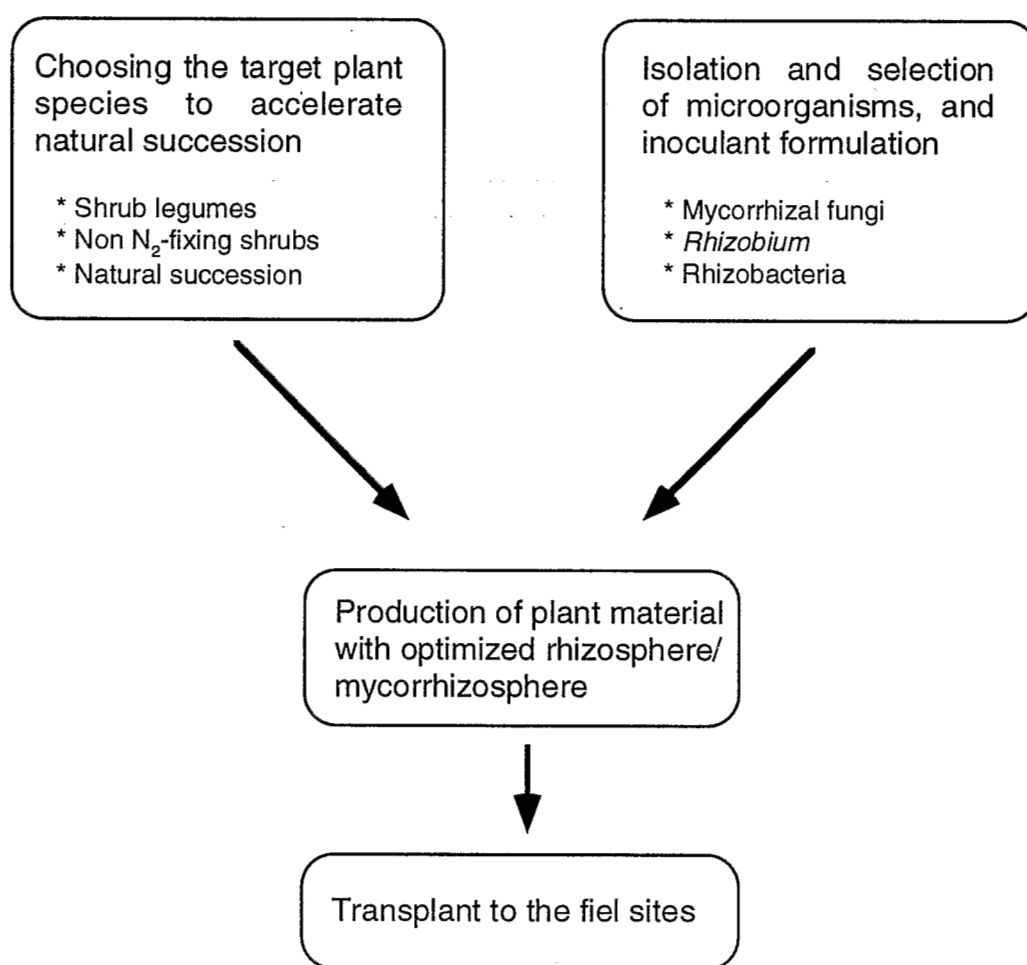


Fig. 3. Microorganisms in revegetation programmes for desertification control. Biotechnological inputs.

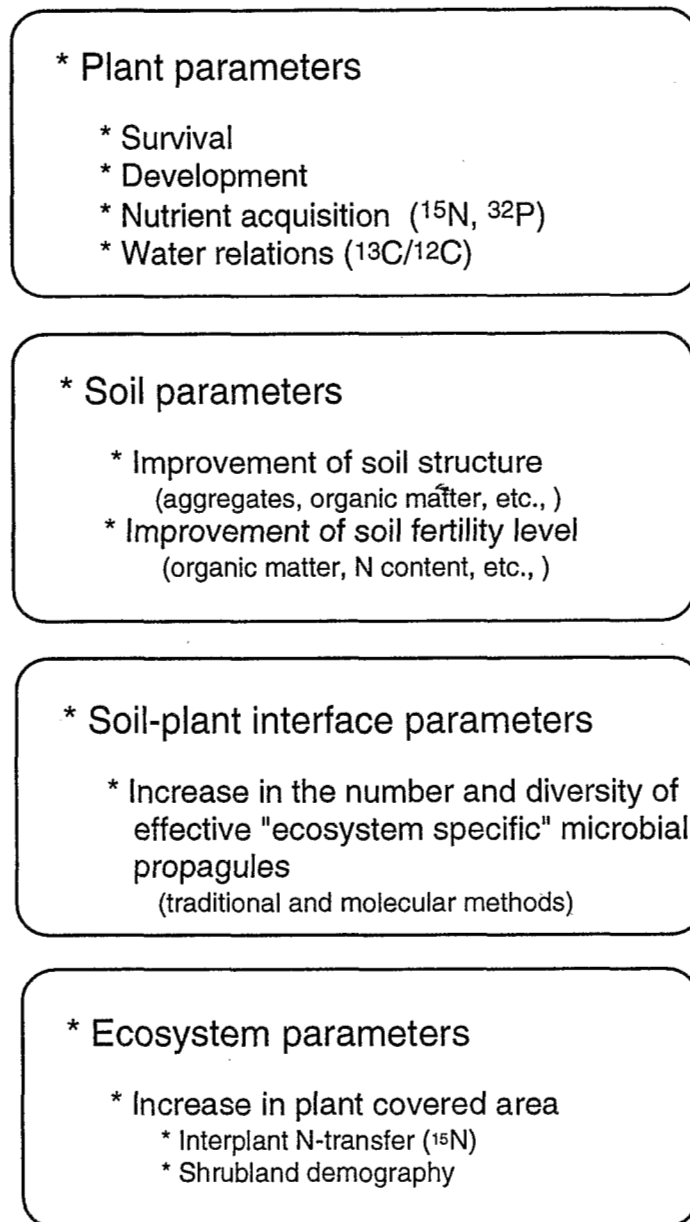


Fig. 4. Microorganisms in revegetation programmes for desertification control. Evaluating the impact of the revegetation on ecosystem rehabilitation.

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