Biol Fertil Soils (2001) 33:118–125

ORIGINAL PAPER

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Microbial populations in heated soils inoculated with cyanobacteria

Received: 8 March 2000

Abstract The composition of soil microbiota in four heated (350 °C, 1 h) soils (one Ortic Podsol over sandstone and three Humic Cambisol over granite, schist or limestone) inoculated (1.5 μg chlorophyll *a* g^{-1} soil or 3.0 μg chlorophyll *a* g^{-1} soil) with cyanobacteria (*Oscillatoria* PCC9014, *Nostoc* PCC9025, *Nostoc* PCC9104, *Scytonema* CCC9801, and a mixture of the four) was studied by cultural methods. The aims of the work were to investigate the potential value of cyanobacteria as biofertilizers for accelerating soil recolonization after fire as well as promoting microbiotic crust formation and to determine the microbial composition of such a crust. The inoculated cyanobacteria proliferated by 5 logarithmic units in the heated soils which were colonized very quickly and, after 2 months of incubation, the cyanobacterial filaments and associated fungal hyphae made up a matrix in which surface soil particles were gathered into crusts of up to 1.0 cm in thickness. These crusts were composed, on average, of 2.5×10^{10} cyanobacteria, 2.8×10^{6} algae, 6.1×10^{10} heterotrophic bacteria (of which 1.2×10^{8} were acidophilic, 1.3×10^{6} were *Bacillus* spp. and 1.5×10^{8} were actinomycetes) and 77.8 m fungal mycelium (1.4×10^{6} were fungal propagules) g^{-1} crust. Counts of most microbial groups were positively correlated to cyanobacterial numbers. The efficacy of treatment depended on both the class of inoculum and the type of soil. The best inoculum was the mixture of the four strains and, whatever the inoculum used, the soil over lime showed the most developed crust followed by the soils over schist, granite and sandstone; however, the latter was comparatively the most favoured by the amendment. In the medium term there were no significant differences between the two inocula rates used. Biofertilization increased counts of cyanobacteria by 8 logarithmic units while heterotrophic bacteria, actinomycetes, algae and fungal propagules rose by >4 logarithmic units, acidophilic bacteria and *Bacillus* spp. by around 3 logarithmic units and fungal mycelia showed an 80-fold increase. The results showed that inoculation of burned soils with particle-binding diazotrophic cyanobacteria may be a means of both improving crust formation and restoring microbial populations.

Keywords Biofertilizers · Crust · Inoculation · Soil reclamation · Fire

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Introduction

Scientific interest in ecosystem degradation as a consequence of fire has increased greatly in the last decade because both the frequency of fires and the size of the burned areas are increasing. Heat dramatically disturbs the soil surface, provokes a decrease in diversity and abundance of soil biota and increases strongly the risk of erosion by wind and water (Ahlgren 1974; Vilariño and Arines 1991; Vázquez et al. 1993; Fritze et al. 1993, 1994, 1998; Bååth et al. 1995; Acea and Carballas 1996a; Prieto-Fernández et al. 1998; Pietikäinen et al. 2000).

Cyanobacteria have a world-wide distribution and can proliferate in habitats with ample fluctuations in environmental parameters as pH, nutrient content and availability, light, aeration, texture, temperature and moisture. In agricultural soils it has been shown that the growth of inoculated cyanobacteria produced significant improvements of some microbiological, biochemical and physical soil properties (Rao and Burns 1990; Rogers and Burns 1994), increased N fertility and yield and N uptake by the crop (Venkataraman 1972; Zimmerman 1993). Cyanobacteria are commonly pioneering species during the amelioration and revegetation of degraded ecosystems, and have been frequently regarded as biofertilizers and soil conditioners (Venkata-

raman 1972; Fogg et al. 1973; Tate and Klein 1985; Rao and Burns 1990; Zimmerman 1993). As CO_2- and N_2-fixers they provide energy and nutrients for the rest of the community and, in addition, cyanobacterial filaments make up a matrix in which associated microbes and soil particles are gathered into what is known as a microbiotic crust.

In burned soils, which suffer a strong alteration of microbiological properties and may recover slowly from surface disturbances (Ahlgren 1974; Fritze et al. 1993, 1994; Pietikäinen and Fritze 1993; Vázquez et al. 1993; Bååth et al. 1995; Acea and Carballas 1996a; Prieto-Fernández et al. 1998), the development of a microbial crust might be critical in maintaining soil stability and normal water and nutrient cycles, to avoid erosion, and subsequent desertification, and to accelerate ecosystem regeneration (St Clair et al. 1986; Greene et al. 1990; Johansen et al. 1993; Eldridge and Bradstock 1994; Eldridge and Greene 1994). Consequently, practices that encourage growth of the crust will ultimately lead to lower levels of environmental degradation, and inoculation with a soil-crust slurry for reclamation of heat-disturbed ecosystems has been proposed (St Clair et al. 1986). Some researchers have also successfully tested amendment with animal manure and plant residues as a means of restoring heat-disturbed soils (Díaz-Raviña et al. 1996; Acea and Carballas 1999).

The aim of the this work was to investigate the potential value of cyanobacteria as biofertilizers for the acceleration of the recolonization of soils after fire and to determine the composition of the microbial community in the cyanobacterial soil crust. The effect of microbial inoculation on crust formation and the changes in autotrophic (cyanobacteria and algae) and heterotrophic (saprophytic bacteria, acidophilic and spore-forming bacteria, actinomycetes and fungi) microbial populations were studied. The importance of the strain and the dose of inoculum used as well as the type of soil were also evaluated.

Materials and methods

Cyanobacterial culture and inocula

Four filamentous diazotrophic cyanobacteria (*Oscillatoria* PCC9014, *Nostoc* PCC9025, *Nostoc* PCC9104 and *Scytonema* CCC9801), isolated from diverse soils, were selected as inocula because of their high growth capacity. Microbes were cultured separately to late logarithmic phase in N- and organic C-free Bold's basal medium (distilled water 1 l, K_2HPO_4 0.08 g, KH_2PO_4 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.08 g, $CaCl_2$ 0.02 g and minor elements solutions 1 ml) (Metting 1994) at 22 °C under illumination (22 W m^{-2}), with cycles of 16 h light and 8 h dark. Cultures were harvested by centrifugation at 8,000 *g* for 10 min at 4 °C, washed twice and resuspended in sterile distilled H_2O before addition to soil.

Soils treatments and incubation

Four different soils were studied: (1) forest (*Pinus pinaster* Aiton) sandy Ortic Podsol over sandstone (SAN), (2) forest (*Querqus robur* L.) sandy loam Humic Cambisol over granite (GRA), (3) forest (*Pinus pinaster* Aiton) silty loam Humic Cambisol over schist (SCH) and (4) pasture (graminaceous) silty loam Humic Cambisol over limestone (LIM). All the soils are from the temperate humid Atlantic European zone (Galicia, NW Spain). The relevant characteristics of the soils are shown in Table 1. Fifty surface (0–15 cm) subsamples of about 1 kg each were collected from each soil. The samples were sieved (< 2 mm) and thoroughly mixed. The soils had $1.5–65.2 \times 10^2$ indigenous cyanobacteria g^{-1} soil and $0.5–9.3 \times 10^6$ heterotrophic microbes g^{-1} soil (Table 1). Subsamples containing 300 g soil each were distributed in thin (< 1 cm) layers and heated at 350 °C in an oven for 1 h; the selected temperature was achieved after 15 min of heating. The obtained black ashes clearly showed morphological signs of the heating similar to those found in the upper centimetres of burned field soils. Portions (100 g) of each soil were separately placed in cylindrical (9 × 9 cm, depth × height) polyethylene containers and thoroughly mixed with 5% of the original fresh soil, to reproduce field conditions and assure microbial diversity (Díaz-Raviña et al. 1992; Acea and Carballas 1999).

Cyanobacteria were inoculated evenly over the entire soil surface by aseptically spreading small volumes of the cyanobacterial suspensions. Five types of inocula were used: (1) *Oscillatoria* PCC9014 (OS), (2) *Nostoc* PCC9025 (N1), (3) *Nostoc* PCC9104 (N2), (4) *Scytonema* CCC9801 (SC), and (5) a mixture of all four (25% of each strain) (MX). Two doses were added: (1) 1.5 μg chlorophyll *a* g^{-1} soil, and (2) 3 μg chlorophyll *a* g^{-1} soil (these quantities are equivalent to 1×10^5 and 2×10^5 cyanobacteria g^{-1} soil, respectively, as well as to 4 and 8 μg chlorophyll *a* cm^{-2}, respectively). Soil moisture was adjusted to 80% of the field capacity by adding distilled sterile water, and the vessels were covered with polyethylene film, randomly distributed, and incubated at 22 °C under illumination (22 W m^2) with periodic cycles of light (16 h) and dark (8 h). The water content was periodically controlled by weighing the vessels and, when necessary, the samples were remoistened. After 2 months of incubation, three replicates of each treatment were withdrawn to carry out determinations of the different variables.

Microbial analyses

These analysis were carried out following standard culture methods for soil microbial populations previously described (Vázquez et al. 1993; Acea and Carballas 1996b). Ten grams of soil surface samples (0–1.0 cm) or crust from each microcosm was diluted 1:10 with sterile distilled water and mixed for 10 min using a magnetic stirrer operating at half speed. The suspension was diluted in tenfold series in 250-ml Pyrex bottles, and five Petri dishes containing solid media or five test-tubes containing liquid media were inoculated with each dilution. The overall microbial population, cyanobacteria and algae, were determined by the most probable number technique, whilst counts of total aerobic heterotrophic bacteria, acidophilic or sporulating bacteria, actinomycetes and fungal propagules were made by the spread-plate method on agar (1.5%). The length of the fungal mycelium was estimated by staining the samples with 0.1% trypan blue and following the membrane filter technique. Cyanobacteria were grown in N- and C-free Bold's basal medium and microalgae in C-free Bold's basal medium; the presence of either cyanobacteria or algae in the positive tubes was confirmed microscopically. To test whether the predominant cyanobacteria in the crusts were the inoculated strains, cyanobacterial taxa were isolated and cultured on solid media for identification by direct microscopy (Rippka et al. 1979). The total heterotrophic population, saprophytic, acidophilic and aerobic spore-forming bacteria (*Bacillus* spp.) were enumerated in yeast-extract medium. Actinomycetes were counted in dextrose-nitrate medium, microscopic techniques being used in case of doubtful colony differentiation, and fungal propagules were counted on Czapeck-Dox medium. All microbes were cultured at pH 7.0 except fungi and acidophilic bacteria which were grown at pH 4.5. The inoculated tubes and plates were incubated at 28 °C for 4–9 weeks and for 7–10 days, respectively.

Table 1 Soil properties (mean ± SD)

	Parent material			
	Sandstone	Granite	Schist	Lime
Unheated				
Microbial counts (g^{-1} soil)				
Total heterotrophs (10^6)	0.5 ± 0.9	1.4 ± 0.6	3.4 ± 0.5	9.3 ± 0.8
Saprophytic bacteria (10^5)	2.4 ± 0.3	5.5 ± 2.1	10.2 ± 1.2	24.2 ± 3.1
Acidophilic bacteria (10^4)	1.0 ± 6.5	6.5 ± 1.1	4.2 ± 0.2	2.1 ± 0.1
Bacillus spp. (10^3)	1.1 ± 0.1	2.2 ± 1.1	2.2 ± 0.2	2.5 ± 0.2
Actinomycetes (10^3)	1.0 ± 0.1	1.1 ± 0.3	1.5 ± 0.2	1.8 ± 0.4
Cyanobacteria (10^2)	1.5 ± 0.2	15.2 ± 1.8	34.2 ± 3.9	65.2 ± 5.2
Algae (10^3)	1.5 ± 0.2	3.4 ± 0.3	5.3 ± 0.4	9.4 ± 0.6
Fungal propagules (10^2)	1.8 ± 0.4	2.8 ± 0.2	1.9 ± 0.4	1.2 ± 0.2
Fungal hyphae (m)	19.1 ± 2.0	33.1 ± 2.6	18.1 ± 1.0	12.1 ± 2.3
Heated				
pH (H_2O)	6.0 ± 0.1	4.7 ± 0.2	5.8 ± 0.1	6.8 ± 0.2
Field capacity (%)	12.5 ± 1.3	48.2 ± 1.6	63.2 ± 2.1	35.9 ± 2.3
Organic C (g kg^{-1} soil)	8.1 ± 1.0	40.2 ± 1.2	16.2 ± 0.1	8.9 ± 0.1
Total N (g kg^{-1} soil)	0.8 ± 0.1	3.9 ± 0.2	2.0 ± 0.0	1.7 ± 0.0
C:N	13	10	8	5
Coarse sand (%)	68.4 ± 3.5	6.2 ± 0.5	3.0 ± 0.1	6.8 ± 0.5
Fine sand (%)	24.5 ± 1.6	58.2 ± 2.3	10.3 ± 0.2	12.1 ± 3.1
Silt (%)	5.7 ± 0.9	12.2 ± 0.3	75.5 ± 3.3	55.0 ± 1.3
Clay (%)	1.4 ± 0.2	23.2 ± 1.8	11.2 ± 0.7	26.1 ± 0.7
Available nutrients				
P (mg kg^{-1} soil)	8.9 ± 1.3	26.6 ± 1.8	45.6 ± 1.9	76.0 ± 3.7
Ca (mg kg^{-1} soil)	158.8 ± 9.5	288.5 ± 9.2	500.3 ± 9.5	1007.0 ± 9.0
Mg (mg kg^{-1} soil)	42.6 ± 4.8	28.7 ± 2.1	98.2 ± 1.2	148.3 ± 2.1
K (mg kg^{-1} soil)	34.3 ± 2.9	34.5 ± 2.3	92.2 ± 3.0	87.3 ± 2.2
Na (mg kg^{-1} soil)	35.2 ± 3.2	48.3 ± 2.6	72.5 ± 4.6	61.5 ± 2.7

Growth techniques such as those described above are able to determine viable microorganisms, reflect biochemical potential of microbiota and are good indicators of gross changes in microbial populations. Most available studies on soil microorganisms, including the references used in the present work, are based on such techniques. However, it must be pointed out that frequently only a small fraction (approximately 1–10%) of the microbial population is estimated by such methods (Torsvik 1990; Zuberer 1994).

Physical and chemical analysis

Organic C was determined by combustion at 900 °C in a Carmograph 12 apparatus with a secondary oven at 400 °C, and total N was determined by Kjeldahl digestion and steam distillation in a Büchi 430 (Tan 1996). The pH was measured in water using a 1:2 soil:water ratio. Field capacity was estimated as the amount of water retained in saturated soil samples at pF 2 (0.1 bar suction). Texture of the soils (International Mechanical Analysis) was determined on air-dried samples separating coarse sand with a 0.2-mm sieve and fine sand and clay by sedimentation. Available Ca, Mg, Na and K were estimated in 0.5 N acetic acid and P in 0.5 M $NaHCO_3$ extracts. Ca and Mg were measured by atomic absorption spectroscopy and K and Na by atomic emission spectroscopy. P was measured by absorption spectrometry at 882 nm.

Statistical analysis

All results are from triplicate determinations and are expressed on the basis of oven dry (105 °C) weight of soil. Before statistical analysis, microbial counts (n_i) were transformed by $X_i = \log(n_i + 1)$ and the SPSS 6.01 statistical package was used to process the data. Tukey's honestly significant difference (HSD) test was used to separate the means and the Pearson correlation coefficient (r) was obtained to evaluate the relationships between the variables. The percentage of data variation attributable to the factors "inoculum" and "soil" was quantified using two-way or one-way ANOVA, the latter in the case of insignificant interaction between the factors.

Results

Uninoculated soils

After addition of 5% of fresh soils and 2 months of incubation, the surface (0–1 cm) layer of the heated soils which were not fertilized with cyanobacteria had an average of 300 cyanobacteria, 400 algae, 7.6×10^6 heterotrophic microbes and 0.9 m fungal hyphae g^{-1} soil (Fig. 1); on these soils no appreciable soil crust was formed. With the exception of acidophilic bacteria and fungi, the soil LIM showed the highest microbial population densities followed by SCH, GRA and SAN.

Inoculated soils

Cyanobacteria were inoculated at two doses (1.5 and 3.0 μg chlorophyll *a* g^{-1} soil) to assess the possible importance of inoculum rate on crust formation and on its cultivable microbial populations. The highest rate seemed to promote a faster initial establishment of cyanobacteria, but in the medium term there were no sig-

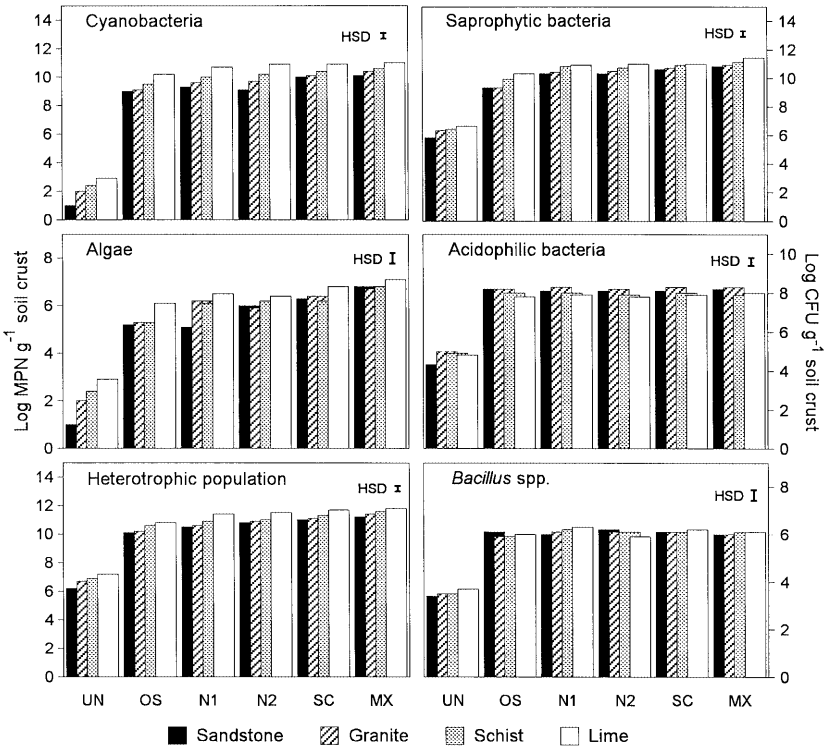

Fig. 1 Microbial populations in the heated soils uninoculated or inoculated with cyanobacteria. *UN* Uninoculated, *OS Oscillatoria* sp., *N1 Nostoc* sp., *N2 Nostoc* sp., *SC Scytonema* sp., *MX*= OS+N1+N2+SC

nificant differences between the two inoculum rates; for this reason, only the results obtained with the lower rate are presented.

Cyanobacteria increased by an average of 5 logarithmic orders and the biofertilized samples had a well-developed microbiotic crust (0.8–1.0 cm thick) with an average of 2.5×10^{10} cyanobacteria g^{-1} soil crust, which represented a mean increase of 8 logarithmic units compared with the surface of the uninoculated soils (Fig. 1). The type of inoculum accounted for a significant amount of the variance in cyanobacterial counts (Fig. 2) and, among the strains used, *Scytonema* sp. showed the highest settlement and growth on all the soils, followed by N1, N2 and OS (Fig. 1). However, the best results were obtained with MX, the average number of cyanobacteria here being up to 8 times higher than with the single-strain inocula. The type of soil also explained a considerable amount of the variance in cyanobacterial counts (Fig. 2) and, irrespectively of the inoculum used, LIM sustained a better cyanobacterial growth (up to 12 times greater average count) than the other soil types, followed by SCH, GRA and SAN (Fig. 1).

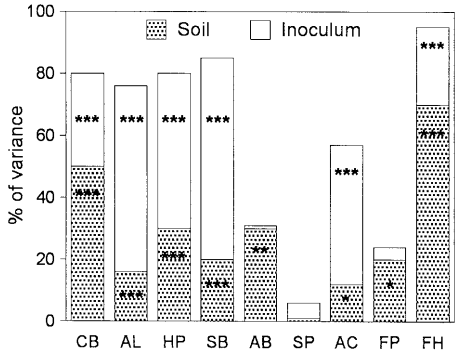

Fig. 2 Percentage of variance of the soil microbiota explained by the type of soil and the class of inoculum. *CB* Cyanobacteria, *AL* algae, *HP* viable heterotrophic population, *SB* saprophytic aerobic bacteria, *AB* acidophilic bacteria, *SP* aerobic sporulated bacteria (*Bacillus* spp.), *AC* actinomycetes, *FP* fungal propagules, *FH* fungal hyphae; $*P\le0.02$, $**P\le0.005$, $***P\le0.001$

A large quantity of algae were present in the crust (average 2.8×10^{6} algae g^{-1} soil crust; Fig. 1), and their counts were correlated to those of cyanobacteria ($r=0.85$, $P\le0.0001$). The type of inoculum explained a high percentage of variance (Fig. 2) and, as in the case of cyanobacteria, MX was more effective (up to 17 times greater) than each strain inoculated separately; the average value of algae in relation to the class of inoculum followed the order MX>SC>N2≥N1>OS (Fig. 1). Algal density was also affected by the soil type, and the rank order was LIM>SCH=GRA≥SAN (Fig. 1). Cyanobacterial inoculation stimulated the proliferation of these autotrophs, the microbiotic crusts contained an average number of algae >4 orders of magnitude higher than that of the control soils.

The microbiotic crusts also contained high heterotrophic populations (average 1.7×10^{11} microbes g^{-1} soil crust; Fig. 1) whose counts were clearly correlated to those of cyanobacteria ($r=0.95$, $P\le0.0001$) and algae ($r=0.90$, $P\le0.0001$). Microbial density was affected by the inoculum used and by the soil type (Fig. 2). MX was up to 11 times more effective in

promoting microbial growth than each inoculum applied separately, the rank order being MX>SC>N2≥N1>OS (Fig. 1). In relation to the type of soil, the rank order was LIM>SCH>GRA>SAN (Fig. 1). In the inoculated samples microbial numbers were >4 orders of magnitude higher than those of the corresponding untreated samples. The positive effect of the cyanobacterial inoculum on the whole microbial population followed the order SAN>LIM>SCH =GRA, the effect on SAN being almost threefold that on the other soils.

Aerobic saprophytic bacteria reached an average of 6.1×10^{10} bacteria g^{-1} soil crust and, among them, 1.2×10^{8} bacteria g^{-1} soil crust were acidophilic and 1.3×10^{6} bacteria g^{-1} soil crust were *Bacillus* spp. (Fig. 1). Bacterial counts were correlated to those of cyanobacteria ($r=0.90$, $P\leq0.0001$), algae ($r=0.87$, $P\leq0.0001$) and total population ($r=0.93$, $P\leq0.0001$). The bacteria able to form spores were not correlated to any studied group while the acidophiles were negatively correlated to both cyanobacteria ($r=-0.60$, $P<0.007$) and the whole heterotrophic population ($r=-0.50$, $P<0.03$). Actinomycetes reached an average of 1.5×10^{8} actinomycetes g^{-1} soil crust (Fig. 3) and were positively correlated to counts of cyanobacteria ($r=0.85$, $P\leq0.0001$), algae ($r=0.90$, $P\leq0.0001$), total heterotrophs ($r=0.94$, $P\leq0.0001$) and total bacteria ($r=0.95$, $P\leq0.0001$). The class of inoculum was highly significant in explaining the variation in bacterial and actinomycetes counts but did not affect those of acidophilic or sporulated bacteria (Fig. 2). MX was more effective with respect to the whole bacterial population (up to 17 times greater) and actinomycetes population (7 times greater) than each inoculum applied separately (Fig. 1, 3), the average number of microbes in relation to the inoculum following the order MX>SC>N2≥ N1>OS (Fig. 1). Except for the bacteria which belong to the genera *Bacillus*, the type of soils affected significantly all the heterotrophic bacterial groups studied (Fig. 2). The order of abundance of total bacteria and actinomycetes was LIM>SCH>GRA≥SAN (Figs. 1, 3), the average counts of LIM were 4 times and twice, respectively, those of the other soils, whilst numbers of acidophilic bacteria in GRA and SAN were twice those in SCH and LIM. Inoculation provoked an average increase of 4.3, 3.3, 2.6 and 4.6 logarithmic orders in total bacteria, acidophilic and sporulated bacteria and actinomycetes, respectively.

Fungi reached an average of 77.8 m mycelium and 1.4×10^{6} propagules g^{-1} soil crust after incubation (Fig. 3). Fungal mycelia and propagules were positively correlated ($r=0.80$, $P\leq0.0001$), the latter being also correlated with acidophilic bacteria ($r=0.51$, $P\leq0.03$). The factors "soil" and "inoculum" affected the length of hyphae in the crusts, whereas the number of propagules was only affected by the soil type (Fig. 2). The length of mycelium in soils inoculated with MX was twice that with the other simpler inocula, the effectiveness being the same as in the case of the bacterial heterotrophic groups (MX>SC>N2≥N1>>OS) (Fig. 3). The order of hyphae length and propagule numbers of the soils were GRA≥SCH>SAN≥LIM, the average values of the first two being twice those of the others (Fig. 3). Biofertilization favoured fungal proliferation; the mycelium and the propagules present in the microbiotic crusts were, respectively, 77 times and 4.4 orders of magnitude higher than those of the uninoculated soil.

The magnitude of the increase in cultivable prokaryotes and algae with respect to the corresponding uninoculated samples (SAN>GRA>SCH>LIM) was, generally, higher in the soils with a smaller original population (Figs. 1, 3), while the positive effect on fungi followed the order GRA≥SCH>SAN≥LIM for mycelia and SAN>SCH=GRA>LIM for propagules (Fig. 3).

Discussion

Heating sterilized the soils, as occurs in the first centimetres of most soils affected by fires. Microbial death

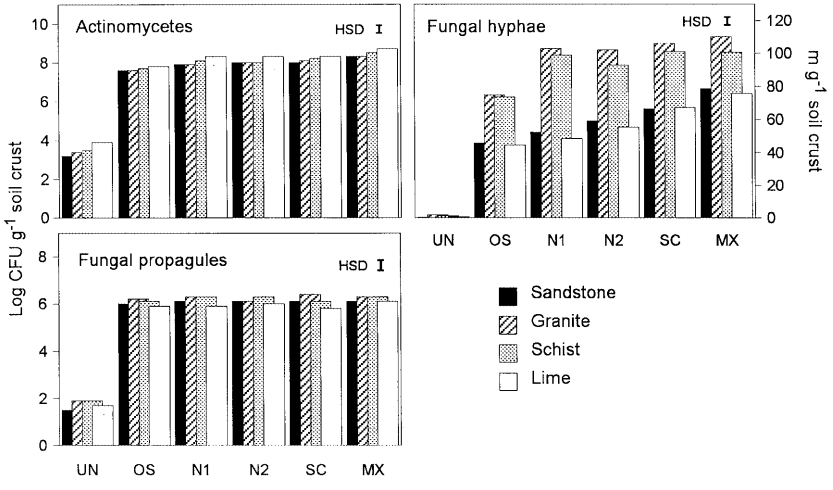

Fig. 3 Actinomycetes and fungi in the heated soils uninoculated or inoculated with cyanobacteria. For abbreviations, see Fig. 1

was probably the cause of the sharp reduction in microbial number, diversity or activity reported by other authors after soil heating or forest fires (Ahlgren 1974; Fritze et al. 1993, 1998; Pietikäinen and Fritze 1993; Díaz-Raviña et al. 1996; Prieto-Fernández et al. 1998). Microorganisms added with the fresh soil were capable of growing, and within 2 months, plate-count saprophytic bacteria predominated with respect to the other microbial groups, followed by fungal propagules and actinomycetes; whereas fungal mycelia and the most probable numbers of photoautotrophs, cyanobacteria and algae, were relatively low. Some authors (Ahlgren 1974; Vilariño and Arines 1991; Fritze et al. 1994, 1998; Bååth et al. 1995; Pietikäinen et al. 2000) have reported a strong alteration of the microbial community structure due to the modification of the soil chemical and physical status by fire. In the uninoculated soils the microbial community did not form an appreciable soil crust. Disruption or even total absence of a cyanobacterial crust is common in many fire-disturbed ecosystems (Greene et al. 1990; Johansen et al. 1993; Eldridge and Bradstock 1994) in which the lack of such cover favours soil erosion (St Clair et al. 1986; Zimmerman 1993; Eldridge and Greene 1994) and alterations in the C and nutrient cycles (Venkataraman 1972; Fogg et al. 1973; Zimmerman 1993; Eldridge and Greene 1994).

The inoculated cyanobacteria were able to actively and extensively proliferate in the soils, which were colonized very quickly and thus, after 2 months, had a higher density than in the unamended soils. At the end of the process, the surface of the soils was covered by a well-developed microbiotic crust (0.8–1.0 cm thick). The ability of cyanobacteria to colonize heated soils has also been reported by Vázquez et al. (1993), who found that indigenous cyanobacteria increased by 3 logarithmic orders 1 year after a wildfire and became the dominant component of the photosynthetic communities in the soil. Other researchers have reported a significantly greater number of cyanobacteria in fire-disturbed soils inoculated with a soil-crust slurry, and showed that total cyanobacterial cover increased with time after fire (St Clair et al. 1986; Greene et al. 1990; Johansen et al. 1993; Eldridge and Bradstock 1994).

The crusts were composed of a mesh of cyanobacterial filaments forming closely inter-twined rope-like bundles in and among the ashes. Besides cyanobacteria, fungal hyphae and algae which may also play an important role in soil stabilization and aggregation, as well as heterotrophic bacteria (a small proportion of which were acidophiles or sporulated) and actinomycetes, were present in the crust. Microbial densities found in the crust were higher than those reported for most soils (Alexander 1967; Skujinš 1984; Acea and Carballas 1990; Vázquez et al. 1993) or for several types of crust of untreated soils (Skujinš 1984; Wheeler et al. 1993), but similar or lower than those found by Rogers and Burns (1994) in an agricultural soil biofertilized with *Nostoc* spp.. Microorganisms in the crust enhance the stability of the soil surface and protect it from wind and water erosion by the physical binding of the filamentous components of the crust as well as by the secretion of amorphous gel-like organic materials (Zimmerman 1993; Eldridge and Greene 1994; Rogers and Burns 1994; Falchini et al. 1996). A microbial crust has also shown to ameliorate the water kinetics of the soil and to aid in plant seedling establishment (Zimmerman 1993; Eldridge and Greene 1994; Rogers and Burns 1994). In addition, the metabolic activity of microbes (i.e. C and N_2 fixation and organic matter mineralization) present in the crusts can also have an important beneficial effect in the burned soils that, usually, have a strong alteration of the nutrient cycles (Ahlgren 1974; Prieto-Fernández et al. 1993, 1998; Fritze et al. 1998; Pietikäinen et al. 2000).

The increase in cultivable microbial populations due to biofertilization was higher than those reported for unheated or heated soils amended with wheat straw, cattle slurry, poultry manure or composted urban refuse (Díaz-Raviña et al. 1989; Acea and Carballas 1996b, 1999). In addition, counts of most microbial groups were positively correlated to cyanobacterial numbers. These results indicate that inoculation with alien cyanobacteria did not negatively affect the growth of the heterotrophic or the autotrophic microbes in the heated soils. In particular, biofertilization seemed to nullify the negative effect of burning or heating on fungi and algae found by some authors (Vázquez et al. 1993; Bååth et al. 1995; Acea and Carballas 1999). In non-burned soils, a stimulatory effect of inoculated cyanobacteria on microorganisms, specially on heterotrophic bacteria, has been observed; this effect was attributed to the carbonaceous and nitrogenous metabolites excreted by the cyanobacteria (Rao and Burns 1990; Rogers and Burns 1994). Except for the major increase in cyanobacterial populations, biofertilization did not change the order of the relative abundance of the various microbial groups studied, which were similar to those found in these and other untreated soils (Acea and Carballas 1990, 1996a, 1996b, 1999; Vázquez et al. 1993). Thus, most heterotrophic microorganisms present in the crust were aerobic bacteria, a small proportion of which were acidophilic or acidotolerant and a lesser number spore forming, followed by actinomycetes and fungal propagules. The fact that bacterial numbers were correlated to those of the whole heterotrophic population and that the biofertilization effect on total microbial density could be somehow extended to saprophytic bacteria was in agreement with the predominance of bacteria in the microbial community.

The efficacy of treatment depended on the type of inoculum. The best inoculum was MX and, among the strains used, SC showed the highest settlement and growth capacity on all the soils. Indeed, in some areas, *Scytonema* sp. is specially common after soil burning and forms a cyanobacterial crust (Lynch 1983), although other researchers also found a high proliferation of *Nostoc* sp. in burned soils (St Clair et al. 1986).

The amount of inoculum affected the initial stages of colonization, which was faster with the higher inoculum rate; nevertheless, in the medium term the proliferation of cyanobacteria cancelled out the differences between the two inocula rates. Similar results were obtained by Rogers and Burns (1994) for non-heated soils.

The type of soil also affected microbial numbers and, regardless of the inoculum used, LIM showed the most developed crust followed by SCH, GRA and SAN. Apart from the probable relationships between soil pH and cyanobacterial proliferation, this result may also be due to the higher content of available P, Ca, Mg, K and other nutrients of LIM and SCH. All these parameters have been demonstrated to strongly affect the development of cyanobacteria (Fogg et al. 1973; Rippka et al. 1979; Reynaud 1987). Cyanobacteria increased as particle size and the C:N ratio decreased, while there was no apparent relationship between cyanobacteria and either organic C or N content. These results indicated that in these heated soils, apart from a possible effect of the alteration of the organic matter by heating (Bååth et al. 1995; Fritze et al. 1998; Prieto-Fernández et al. 1998; Pietikäinen et al. 2000), that texture and nutrient content are factors that considerably affected the microbial populations, as is also the case in non-heated soils. The increase in microbes as compared to the uninoculated samples was higher where the original soil populations were lower, probably because the beneficial effect of the inoculum and the development of a microbial crust was more important in soils with higher nutrient limitation and smaller microbial populations.

In conclusion, these results showed that inoculation of burned soils with particle-binding diazotrophic cyanobacteria may be an ecotechnological means of both improving crust formation and restoring microbial populations. These autotrophs can survive and rapidly grow in heated soils, favouring the proliferation of other microorganisms, even of those that usually are the most disfavoured by heating. Cyanobacteria, because of their morphology (filamentous structure) and/or physiology (secretion of polysaccharide and other cementing substances, autotrophy and N_2-fixing capacity), generate a suitable environment in which other microbial populations can proliferate and form a microbial crust, whose role in preventing soil erosion, improving water kinetics and plant seedling is well known. Such crusts were not present in the uninoculated soils. A relatively low rate of inoculation is sufficient for the development of soil crusts, but the effectiveness of biofertilization depends on both the type of soil and the class of inoculum.

Acknowledgements The authors are most deeply indebted to Rosa Nodar for her collaboration in the isolation and identification of the bacterial strains. We are grateful to Cristina Cuiña and José Salmonte for their kind technical assistance. Funding for this research was provided by the Consellería de Educación de la Xunta de Galicia in Santiago de Compostela and by the Comisión Interministerial de Ciencia y Tecnología (CICYT) in Madrid.

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