Evaluation of legumes and poultry manure for the early protection of burnt soils

Evaluación de leguminosas y gallinaza para la protección temprana de suelos quemados
Avaliação da utilização de leguminosas e estrume de galináceos na protecção precoce de solos queimados

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ABSTRACT

Organic amendments combined with the sowing of gramineous grasses are effective for the early protection of burnt soils (BS) but cannot restore soil N status to pre-fire level; this has led to interest in combining their use with N_2 fixer legumes. The effectiveness of applying poultry manure (PM; 2 Mg ha^{-1}) and sowing legumes (*Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium repens*) for the early protection of BS was compared with that of applying PM + *Lolium perenne* and growing these four species without PM in a 3-month pot experiment, which also included a control consisting of an unburnt soil (US). In US, the shoot and root biomass increased as follows: *Trifolium ~ Lotus << Lolium < Lupinus*. Compared with those grown in US, plants grown in BS were smaller and weaker in three species (*Lupinus, Lolium* and *Trifolium*). The reverse was true for the four species grown in BS+PM, which showed the benefits of PM addition. In all the treatments, plant N uptake, which prevents soil-N losses, increased as follows: *Trifolium ~ Lotus < Lupinus < Lolium*. The lack of nodules observed suggested that none of the legumes fixed atmospheric-N_2.

RESUMEN

Las enmiendas orgánicas combinadas con la siembra de gramíneas son efectivas para la protección temprana de los suelos quemados (SQ) pero insuficientes para restaurar el estatus pre-incendio del N-edáfico, lo cual deriva en el interés de emplear dichas enmiendas en combinación con leguminosas fijadoras de N_2. La utilidad de la gallinaza (G, 2 Mg ha^{-1}) y la siembra de leguminosas (Lotus corniculatus, Lupinus polyphyllus y Trifolium repens) para la protección temprana de SQ se comparó con la de G + Lolium perenne y esas cuatro especies sin G en una experiencia en invernadero durante tres meses que también incluyó un suelo control no quemado (NQ). En NQ, la biomasa de tallos y raíces aumentó en el orden Trifolium ~ Lotus << Lolium < Lupinus. Comparadas con las de NQ, las plantas de SQ fueron más pequeñas y débiles en tres especies (Lupinus, Lolium y Trifolium), mientras que en las cuatro especies ensayadas la biomasa aérea y subterránea fue mayor en SQ+G, demostrando los beneficios de la adición de gallinaza. En todos los tratamientos, la asimilación de N por las plantas, que previene las pérdidas de N del suelo, aumentó en el orden Trifolium ~ Lotus < Lupinus < Lolium. La ausencia de nódulos sugiere que ninguna de las leguminosas fijó N_2 atmosférico.

RESUMO

A aplicação combinada de materiais orgânicos com gramíneas tem demonstrado ser eficaz na protecção precoce de solos queimados (SQ), embora não suficientemente eficaz para restaurar o "status" inicial de N no solo antes da ocorrência de incêndios florestais. Assim, parece justificar-se a aplicação combinada de materiais orgânicos com culturas leguminosas fixadoras de N atmosférico. Num estudo, conduzido em estufa durante 3 meses, com o objectivo de protecção precoce de um solo queimado (SQ), comparou-se o uso de estrume de galináceos (G, 2 Mg ha^{-1}) em combinação com as leguminosas (Lotus corniculatus, Lupinus polyphyllus y Trifolium repens), com G + Lolium perenne e ainda o uso dessas quatro espécies sem G. Como controlo foi usado um solo não queimado (NQ). No NQ verificou-se um aumento de caules e raízes seguindo a ordem Trifolium ~ Lotus << Lolium < Lupinus. Comparadas com as NQ as plantas de SQ apresentavam um porte inferior e eram mais frágeis nomeadamente no caso de três espécies (Lupinus, Lolium y Trifolium), enquanto que para as quatro espécies ensaiadas a biomassa aérea e subterrânea foi maior em SQ+G demonstranto os benefícios da adição de estrume de galináceos. Para todos os tratamentos, a absorção do N pelas plantas, que previne perdas de N do solo, aumentou segundo a ordem Trifolium ~ Lotus < Lupinus < Lolium. A ausência de nódulos sugere que nenhuma das leguminosas fixou N$_2$ atmosférico.

1. Introduction

Wildfires are one of the most widespread and severe factors that degrade terrestrial ecosystems by triggering important losses of soil and nutrients (Pinaya et al. 2000; Thomas et al., 2000; De Luis et al. 2003). The increased post-fire soil erosion risk, closely related with the proportion of bare soil (Vega et al. 2005), continues to be a threat until vegetation cover has recovered (Taskey et al. 1988). As wildfires can drastically reduce the soil seeds bank, natural re-vegetation of burnt soils is delayed allowing to losses of ashes and soil (Chandler et al. 1983; Casal 1992; Faraco et al. 1993; Vázquez et al. 1996). Therefore, soil re-vegetation must be carried out as soon as possible after wildfire to reduce soil damage (Vázquez et al. 1996; De Luis et al. 2003). Due to its beneficial effects on key soil characteristics (soil aggregation, microbial mass and activity, nutrient cycles, stabilization of the ash layer, etc.) and on the recovery of the vegetation cover, organic amendments combined with gramineous plant sowing has been widely considered as a promising technique for the early protection of burnt soils (Vázquez et al. 1996; Villar et al. 1998; Castro et al. 2000; Guerrero et al. 2001; Meyer et al. 2004; Villar et al. 2004; Villar et al. 2005). Among organic amendments, Villar et al. (1998) found better plant growth rates in a burnt soil amended with poultry manure as compared with cattle slurry or sewage sludge; moreover, Castro et al. (2000) reported that poultry manure greatly enhanced gramineous plant growth even at low doses (1-4 Mg ha^{-1}) enough to ensure its technical, economical and ecological applicability on burnt forest ecosystems. However, this technique is insufficient to recover the pre-fire status and distribution of soil organic N (Castro et al. 2007).

Both Papavassiliou and Arianoutsou (1993) and Vázquez et al. (1996) have reported that ash layer conditions of burnt soils do not inhibit nodule formation in legumes. According to Hendricks and Boring (1999), atmospheric N$_2$ fixation by herbaceous legumes could counterbalance the losses of soil N due to wildfires because these plants are not harvested and their biomass will be incorporated to the soil organic matter. Therefore, the use of herbaceous legumes in post-fire soil revegetation could help to restore the N pool in burnt ecosystems. Many legumes are fire tolerant, or even pyrophytes as *Genista scorpius*, *Cytisus scoparius*, *Ulex europaeus* and *Ulex parviflorus* (Vélez 1986; Moritz and Svihra 1998; López Vila 2003), and their persistence in annually burnt grasslands suggests that they are an important source of N to the ecosystem (Towne and Knapp 1996). Moreover, in regularly burned areas the distribution and diversity of legumes are higher than in areas less frequently affected by fires (Towne and Knapp 1996; Hainds et al. 1999). The presumably high post-fire soil N availability could limit N$_2$-fixation activity, especially in legume seedlings which, unlike the resprouts, do not have immediate access to belowground stored carbon in coarse roots (Casals et al. 2005). Both seedlings and resprouts acquired most of their N from the atmosphere when growing in a soil with moderate N content and availability (3.9 g kg^{-1} soil in the top 5 cm and 46-58 mg N kg^{-1} soil mineralized under field conditions during the first 9 months after fire, see Casals et al. 2005); however, evidence for these results to be generalized to commercial legume species and/or burnt soils with high levels of inorganic and total N is still lacking.

The aim of this research was to evaluate the usefulness of poultry manure amendment combined with commercial herbaceous legumes for the protection and recuperation of a burnt soil with high N content and availability. Three legumes well adapted to soils and climatic conditions of the temperate humid region were selected: *Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium repens*; moreover, the experimental design also includes *Lolium perenne* as a reference species due to its wide use in similar experiments.

2. Materials and Methods

Soil samples

Unburnt soil samples were taken from the A horizon (0-15 cm depth) of a sandy, base desaturated Umbrisol over granite under *Pinus pinaster* Aiton located at Salgueiras Hill (Galicia, NW Spain), where the mean annual temperature and precipitation are 13.7 °C and 1 350 mm, respectively, and there were not recent fires (preceding 20 years). Six samples, which represented a total of 240 kg of soil, were taken at random from a surface area of 1 000 m^2, mixed to obtain a composite sample, sieved at 4 mm and thoroughly homogenized. The unburnt soil was acidic (pH_{KCl} 4.0), contained 66.6 g C kg^{-1} soil and 4.31 g N kg^{-1} soil. Soil was heated at 385 °C for 10 min in a laboratory furnace (preheated to this temperature) under programmed conditions simulating those of high severity wildfires (Fernández et al. 1997).

Poultry manure

Poultry manure (PM) was obtained from an industrial farm, collected at random in plastic bags 3 days before the beginning of the experiment and kept in a refrigerator at 4 °C. Before use, poultry manure was thoroughly homogenized and, after removing big feathers, subsamples were slightly chopped and they were sieved (<4 mm) and homogenized again. Poultry manure contained 3.45% of total N and 0.44% of inorganic N.

Vegetative pot experiment

The experiment was run in a greenhouse, under natural illumination. Pottery pots (17 cm diameter, 14 cm high, 227 cm^2 surface) were filled with 1 kg of dry soil and twelve treatments were set up: 3 soil treatments (unburnt soil, US; burnt soil without poultry manure, BS; and burnt soil with a dose equivalent to 2 Mg ha^{-1} of dry poultry manure, BS+PM) and 4 plant species (*Lotus corniculatus*, *Lupinus polyphyllus*, *Trifolium repens* or *Lolium perenne*). Three replicates per treatment were made. The poultry manure dose was that recommended by Castro et al. (2000) according to cost to benefit criteria. In each pot, 117 seeds were sown without pregermina-

tion and covered with a 0.5 cm soil layer, previously mixed with the poultry manure dose in the BS+PM treatments. All pots were brought to 75% of soil water-holding capacity and watered gravimetrically to this moisture level every 1-2 days, as necessary. After 3 months of growing (September-November) shoots and roots were separately cropped, dried at 60 °C, weighed and finely ground (50-100 mm) in a stainless steel ball mill.

Physical and chemical analyses

The dry matter content of soils, poultry manure and plants was assessed by oven-drying fresh material at 110 °C for 5 h. Soil water-holding capacity was determined in a Richards membrane-plate extractor at a pressure corresponding to a matrix potential of 10 kPa.

The plant and soil total N was measured on finely ground samples with an elemental analyser (EA). Reliability of the total N analyses was verified by checking a certified standard (Euro-Vector S.p.A., Milano, Italy) in every set of 10 analyses.

Statistical analyses

Data were statistically analysed by two-way ANOVA (with plant species and soil as factors) and the Levene's test was used for verifying the equality of variances among groups. In the case of homocedasticity, significant differences among the mean groups were established at p<0.05 using the Bonferroni's test for multiple comparisons. Otherwise, the original data were subjected to Cox-Box transformations and then significant differences among the mean groups were established at p<0.05 using the Bonferroni's test for multiple comparisons. Statistical procedures were performed with SPSS 15.0 for Windows.

3. Results

Irrespectively of the plant part considered (shoots, roots or whole plant), the two-way ANOVA showed very significant (P<0.001) effects on phytomass production due to soil treatment (partial η^2 = 0.832 to 0.864), plant species (partial η^2 = 0.976 to 0.982) and the interaction between the two factors (partial η^2 = 0.887 to 0.938). In the unburnt soil (US) the biomass of shoots, roots and the whole plant at the end of the experiment (**Figure 1**) increased in the order *Trifolium ~ Lotus << Lolium < Lupinus*, differences being significant (P<0.05) except between the two first species. Compared with US, in the burnt soil (BS) the plant growth was significantly lower in the case of *Lupinus* and not significantly in that of *Lolium* and *Trifolium*, whereas *Lotus* growth was slightly, but not significantly, higher. The addition of poultry manure (BS+PM treatment) was beneficial for the four species tested in the present experiment, although plant growth was significantly enhanced only for *Lupinus* and *Lolium*, especially in the latter (more than 100%), compared with BS. Consequently, at the end of the experiment the biomass in the BS+PM treatment increased significantly in the order *Trifolium ~ Lotus<< Lupinus < Lolium*.
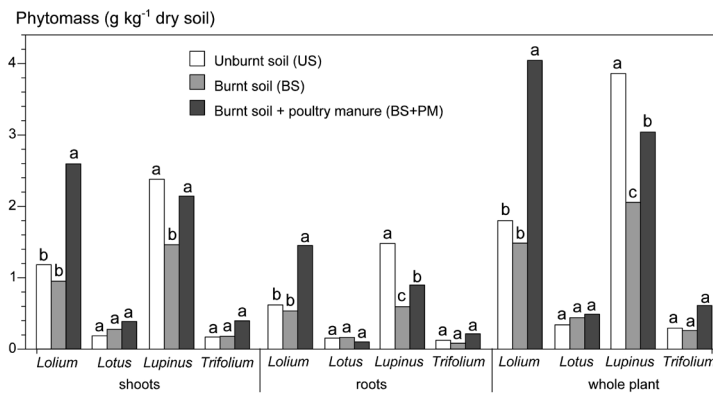

Figure 1. Biomass production, and distribution between shoots and roots, for *Lolium perenne*, *Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium repens* from the unburnt soil and the burnt soil with and without poultry manure addition. For each species and plant part (shoots, roots, whole plant), different letters (a, b, c) indicate statistically significant differences among soil treatments (p<0.05).
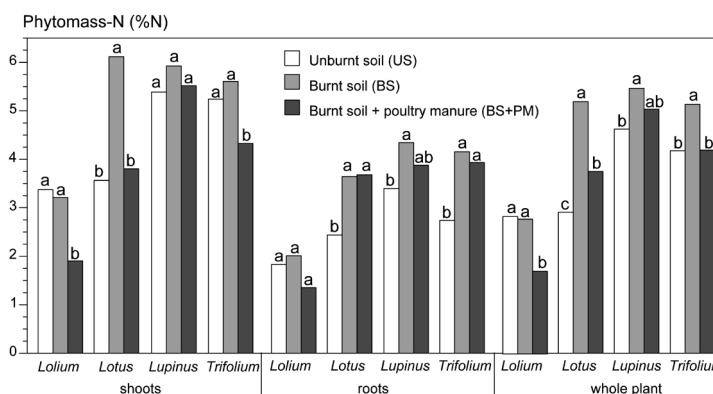

Figure 2. N concentration in shoots, roots and the whole plant of *Lolium perenne*, *Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium repens* from the unburnt soil and the burnt soil with and without poultry manure addition. For each species and plant part (shoots, roots, whole plant), different letters (a, b, c) indicate statistically significant differences among soil treatments (p<0.05).
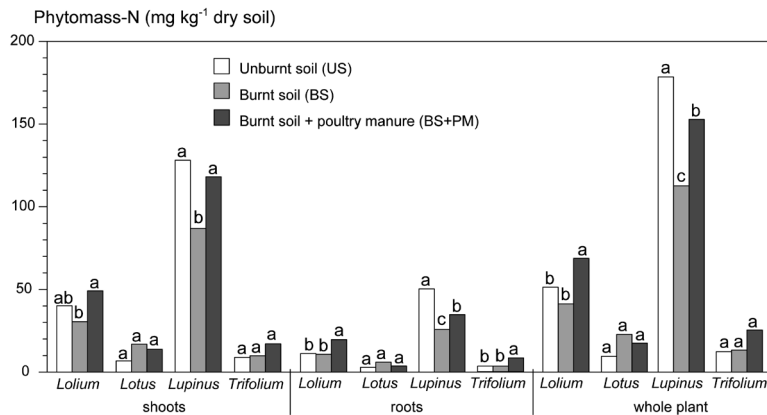
Figure 3. Amount of N in shoots, roots and the whole plant of *Lolium perenne*, *Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium repens* from the unburnt soil and the burnt soil with and without poultry manure addition. For each species and plant part (shoots, roots, whole plant), different letters (a, b, c) indicate statistically significant differences among soil treatments (p<0.05).

As for phytomass production, the two-way ANOVA showed significant (P<0.001) effects on plant N concentration (shoots, roots or whole plant) due to soil treatment (partial η^2 = 0.624 to 0.729), plant species (partial η^2 = 0.883 to 0.918) and the interaction between the two factors (partial η^2 = 0.484 to 0.670; P<0.01 in the case of roots). Regardless the part of the plant considered and the soil treatment, the concentration of N in the biomass of *Lolium* (**Figure 2**) was significatively (P<0.05) lower than that of the three legumes tested, excepting *Lotus* in the US treatment. Compared with the legumes grown on US, those developed on BS had a higher N content, although the differences were not always significant; conversely, the N concentration in *Lolium* tissues hardly changed from US to BS. Except for *Lotus* roots, the N richness in the legumes decreased (not always significatively) from BS to BS+PM, returning usually to values similar to those of the US plants; in *Lolium*, the concentration of N also decreased from BS to BS+PM, although, unlike the legumes, the values reached in the shoots and the whole plants were significantly lower than those of *Lolium* plants grown on US. The plant biomass yield and the percentage of N in the plants were highly and negatively correlated (r = -0.910, P<0.001; r = -0.883, P<0.002; r = -0.712 and r = -0.686, P<0.05, for the aerial parts of *Lolium*, *Trifolium*, *Lotus* and *Lupinus*, respectively).

For the total amount of N in all plant parts (shoots, roots or whole plant), the two-way ANOVA also showed significant (P<0.001) effects due to soil treatment (partial η^2 = 0.518 to 0.722), plant species (partial η^2 = 0.982 to 0.986) and the interaction between both factors (partial η^2 = 0.688 to 0.913). Either in US, BS or BS+PM, the absolute amount of N contained in the whole plant (**Figure 3**) increased significantly in the order *Trifolium* < *Lolium* < *Lupinus*, with *Lotus* in an intermediate position between the first two species. However, it should be highlighted that a substantial part of the legumes-N (up to 20-30% in *Trifolium* and *Lotus*; around two-thirds in *Lupinus*; data not showed) could not proceed from the soil, but from the N-rich seeds of these species. The pools of shoot- and root-N in US and BS+PM followed the same trend as that of the whole plant, whereas in BS the differences among *Lupinus*, *Lolium* and *Lotus* were not statistically significant. No visible nodules were found at harvesting in the roots of any legume plants.

4. Discussion

The lower plant growth in the burnt soil (BS) than in the unburnt control (US) agrees with the limited growth of the commercial plant species used for the reclamation of burnt soils found elsewhere (Villar et al. 1998; Castro et al. 2000); as the decrease of plant growth was significant only for *Lupinus*, this was the species most negatively affected by the effects of fire on soil. In the burnt soil amended with poultry manure (BS+PM), the improved plant growth agrees with the results reported elsewhere for this and other organic amendments (Villar et al. 1998; Castro et al. 2000; Guerrero et al. 2001; Meyer et al. 2004; Villar et al. 2004, 2005). Villar et al. (1998) showed that the lower plant growth in BS than in BS+PM was not due to macronutrients (N, P, K, Ca, Mg) shortage in the former; these authors hypothesized that biomass production in BS could be inhibited by heat-derived phyto-toxic compounds (see Rovira and Bowen 1966; Raison 1979; Vilariño and Arines 1991; and Díaz-Raviña et al. 1996), their negative effects being microbially or chemically suppressed by the addition of organic wastes.

The negative correlation between the plant biomass and percentage of N agrees with the decrease in plant N content as the crop mass increases, as reported by Zagal (1994) and Lemaire and Gastal (1997). According to Lemaire and Gastal (1997), these results showed that the plants from BS, with reduced size and higher N richness, have a lower proportion of struc-tural vs. metabolic tissues than plants grown on BS+PM. As previously indicated by Castro et al. (2000), the soil conditions in the BS treatment led to weaker plants that cannot provide pro-tection for a recently burnt soil against erosive forces, unlike plants grown in BS+PM.

The ability of plants to retain the post-fire pulse of available N within the soil-plant system, thus preventing N losses by leaching and erosion, varied among species, increasing in the order *Trifolium, Lotus < Lolium < Lupinus*; however, the last two species could interchange their po-sitions taking into account the N they obtained from their seeds. This result has important practical implications for burnt soil reclamation (Castro et al. 2000) because, by reducing the

mineralizable organic-N reserves and increasing the N mineralization rate, wildfires could lead to a rapid depletion of the labile organic N pool (Prieto-Fernández et al. 1993).

No visible nodules were found at harvesting in the roots of any legume plants, a foreseeable result considering the small size of the speci-mens. Therefore, after their senescence and incorporation into the soil organic mater, these plants will not only enrich the soil with fixed N_2. Our results on the lack of legume nodulation contrast with those reported by several authors (Towne and Knapp 1996; Hendricks and Boring 1999; Casals et al. 2005), even with the results of Vázquez et al. (1996) who found that poul-try manure did not inhibit legumes nodulation, which was very much enhanced in the case of *Lotus*. A possible explanation for this differ-ence is that all these authors, except the latter ones, worked on the natural recovery of legume species preexistent in the soil before the fire. Other possible causes for the lack of nodulation of the legumes in BS are that they were com-mercial species that: a) although relatively fru-gal and characteristic of unfertile soils (acidic, sandy, saline), could not be adapted to grow in a burned soil; and b) could be unable to nodu-late due to the lack of the specific symbionts in the soil. Moreover, nodulation and biological fixation of atmospheric N_2 are usually inhibited when there is abundant available N (Macduff et al. 1996; Crews 1999), which is the case of the soils used in the present experiment. Among the ecological constraints that may limit the success of N_2-fixing plants, other than the en-ergetic cost of N fixation, Crews (1999) include the availability of soil nutrients other than N (es-pecially P or Mo) and the existence of adverse edaphic conditions such as high acidity, alkalin-ity or aridity.

Jointly considered, the results on growth, abil-ity to retain the post-fire pulse of available N and absence of N_2 fixation at the short-term suggested that commercial legumes are not as useful as gramineous plants for the very early soil protection and re-vegetation phase of burnt soils. However, as legumes are usually irre-placeable at the medium term for the recovery

of the burnt soil N pool and dynamics, it should be considered the utility of sowing a mixture of legumes (especially *Lupinus*) and gramineous species after the fires.

5. Conclusions

1. In the burnt soil, plant growth was better for *Lolium* than for the three legumes tested, regardless of whether poultry manure was added; this was beneficial for the four species tested.

2. *Lolium* and *Lupinus* can retain the post-fire pulse of available N within the soil-plant system, thus preventing N losses due to leaching and erosion.

3. The combination of applying poultry manure and sowing *Lolium* may offer the best technique for securing immediate post-fire protection of the soil and its nutrients against erosion and leaching; nevertheless, the combined sowing of *Lolium* and *Lupinus* could offer the most useful medium-term solution.

6. Acknowledgements

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