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Utilization of Microbial Inoculation and Compost for Revitalization of Soils

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Abstract: Improving the quality of reclaimed soils requires an active population of microorganisms which can promote plant growth. Increasing the activity of microorganisms can be done by adding nutrients, making agrotechnical soil improvements and by the inoculation of beneficial microorganisms. We investigated the role of fertilizer treatments on plant growth and nitrogen fixation in a pot experiment conducted under green house conditions. Influence of the fertilizer type on numbers of bacteria was also investigated. The seeds were inoculated with the mixture of *Azotobacter* spp. and *Rhizobium* spp. The pot experiment was set up with the substrate from the mine spoil (North Bohemia coal basin, the Czech Republic) using *Medicago sativa* as test plants. The following treatments were used: compost 0, 20, 40, 120, 400, 800 t/ha and mineral fertilizer – ammonium sulphate. The doses of ammonium sulphate were calculated to be equivalent (in nitrogen content) to those doses of compost. Control variants without bacteria inoculation and fertilizers were also included. Inoculation with the mixture of *Azotobacter* spp. and *Rhizobium* spp. significantly increased plant growth and nitrogenase activity. The nitrogenase activity was inhibited by mineral fertilizers in all doses used. The results of the study have proved that compost application stimulated the growth of *Azotobacter* spp. and *Rhizobium* spp.

Keywords: soil revitalization; *Rhizobium* spp.; *Azotobacter* spp.; compost

The evolution of stable and productive soil on the mine spoils requires active microbial populations for effective energy flow and nutrient cycling. This can be achieved either by introducing beneficial microorganisms by way of inoculation or by increasing the microbial activity by the incorporation of amendments (Rao & Tak 2001).

The inoculation by *Rhizobium* spp. is known to increase nodulation, nitrogen uptake, growth and yield response of crop plants (Rudesh *et al.* 2005). Biological nitrogen fixation is an important component of sustainable agriculture, and rhizobial inoculants have been applied frequently as biofertilizers (Sessitsch *et al.* 2002). Much attention has been paid to combined inoculation. The effort to combine several positive effects in one inoculant is understandable because costs remain basically the same, but the effect may be manifold. Therefore, many studies address combined inoculation by the genera *Azotobacter* and *Rhizobium* (Mikanová & Kubát 2006). *Azotobacter* species are free-living, aerobic heterotrophic diazotrophs that depend on an adequate supply of reduced C compounds such as sugars for energy (Kennedy *et al.* 2004).

Another way of biological revitalization of anthropogenic substrates consists in applying various types of organic matter.

The surface of the mine spoil is mostly formed by Miocene clays with unfavorable physical and chemical properties. The properties of these soils (particle size

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distribution, pH, sorption capacity, etc.) can greatly vary in dependence on the properties of the soil substrate from which they are formed (Rohošková *et al.* 2006). Modification is needed prior to biological reclamation. For such purposes, an application of different organic materials is frequently used, such as compost and compostable substrates.

We investigated the role of the compost and mineral fertilizer treatments on plant growth and nitrogen fixation in a pot experiment conducted under green house conditions. We were testing inoculation with nitrogen fixing bacteria *Azotobacter* spp. and *Rhizobium* spp. in reclaimed soils composed mainly of grey miocene clays. One of our aims is the development of an optimal regime of microbial inoculation and fertilization for the establishment of stable vegetation cover. The model plant was *Medicago sativa*. Influence of the fertilizer type on numbers of bacteria *Azotobacter* spp. and *Rhizobium* spp. was also investigated.

MATERIAL AND METHODS

The pot experiment was set up with the substrate from the mine spoil Merkur (North Bohemia coal basin, the Czech Republic) using *Medicago sativa* as test plants. Substrate was composed mainly of grey Miocene clays. The seeds were inoculated with the mixture of *Azotobacter* spp. and *Rhizobium* spp. in the ratio 1:1. The inoculant contained 10^6 CFU of three strains of *Azotobacter* spp. and 10^6 CFU of three strains of *Rhizobium* spp. in 1 ml of liquid suspenzion.

Azotobacter spp. (A001, A006 and A007) and *Rhizobium* spp. (D494, D528 and D557) strains have been maintained in the Rhizobium Collection at the Crop Research Institute – Ruzyně (Kabátová 2006).

The following treatments were used: compost 0, 20, 40, 120, 400, 800 t/ha and mineral fertilizer – ammonium sulphate. The doses of ammonium sulphate were calculated to be equivalent (in nitrogen content) to those doses of compost. Control variants without bacteria inoculation and fertilizers were also included. Some basic characteristics of the compost: The total N content – 1.54%, C_{ox} – 17.9%, C/N – 11.6 and pH (H_2O) – 6.98.

Symbiotic N_2-fixation was measured by means of nitrogenase activity by the acetylene (C_2H_2) reduction technique (Hardy *et al.* 1968), using gas chromatography (Perkin Elmer F30).

The number of *Azotobacter* spp. was determined by means of a standard dilution method and growing on Ashby agar. The number of *Rhizobium* spp. was determined by means of a standard dilution method and growing on pea agar. The number of colony forming unit (CFU) per 1 gram of soil was determined.

Analyses were performed in six replications and average values are presented. All data were processed by one-way analysis of variance (ANOVA) followed by the Tukey HSD test that evaluates the

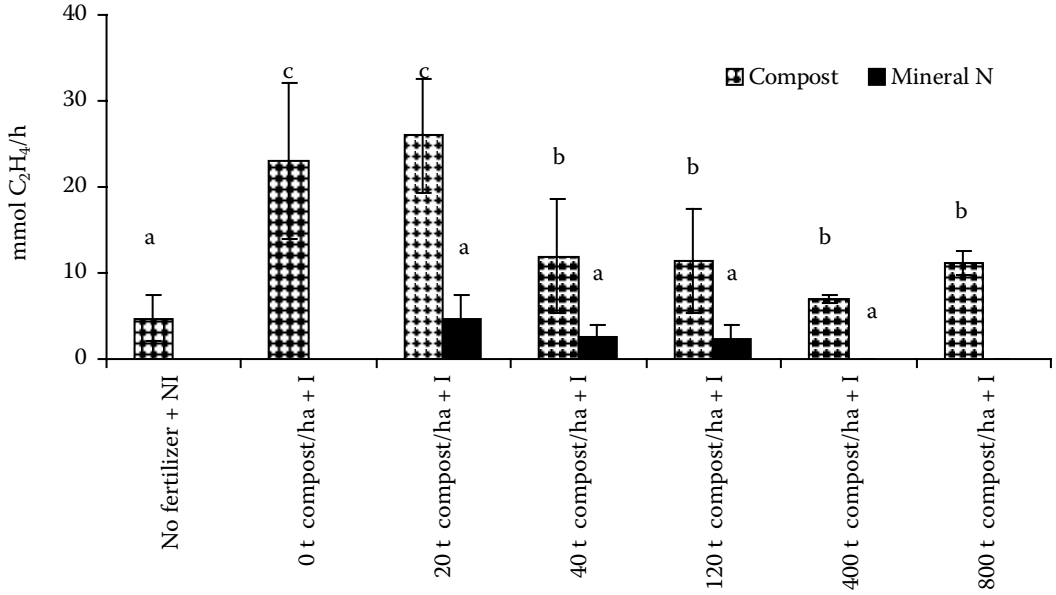

Figure 1. Nitrogenase activity; the columns designed by the same letter do not differ significantly ($P = 0.05$)
NI – no inoculated; I – inoculated with *Azotobacter* spp. and *Rhizobium* spp.

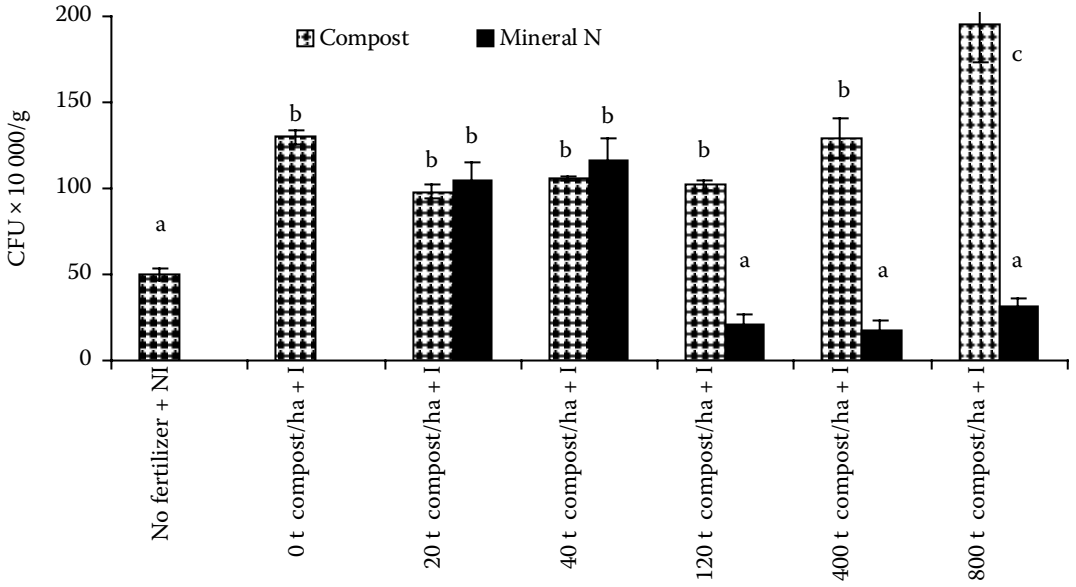

Figure 2. Colony forming unit (CFU) of *Rhizobium* spp. in 1 gram of soil; the columns designed by the same letter do not differ significantly (P = 0.05); NI – no inoculated; I – inoculated with *Azotobacter* spp. and *Rhizobium* spp.

significance of differences among the variants. The columns designed by the same letter do not differ significantly (P = 0.05).

RESULTS AND DISCUSSION

The results of the nitrogenase activity are shown in Figure 1. The inoculation statistically significantly increased the nitrogenase activity. The highest nitrogenase activity was found in the variant without fertilization and in the variant with 20 t of compost per hectare. The nitrogenase activity was inhibited by mineral fertilizers in all doses used. The same result was also reported by Hartley and Schlesinger (2002) and Wu *et al.* (2005).

The inoculation increased the numbers of *Rhizobium* spp. (Figure 2). Compost in doses 800 t/ha

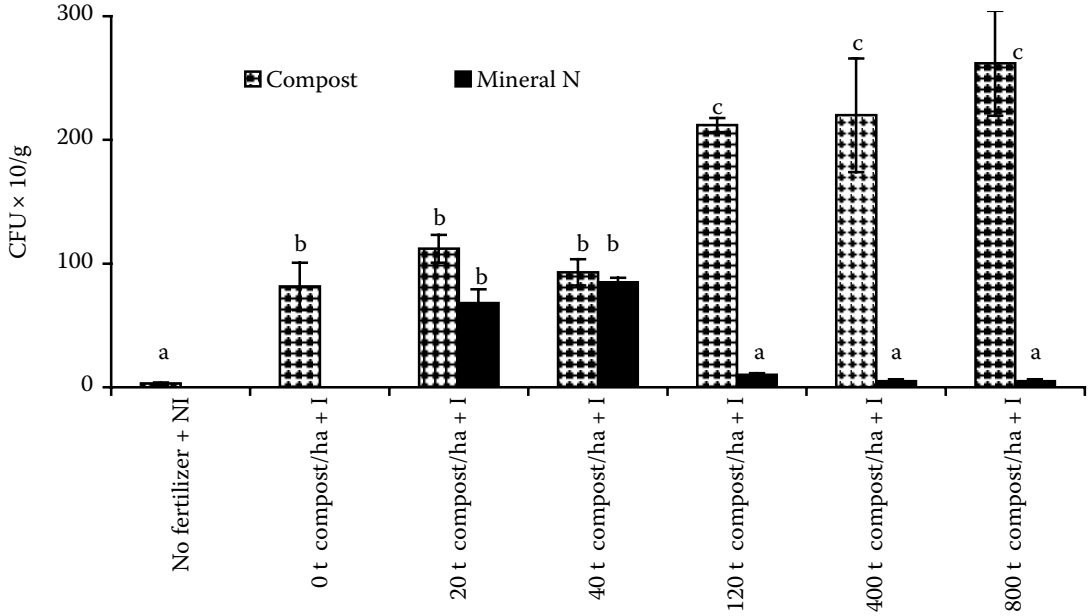

Figure 3. Colony forming unit (CFU) of *Azotobacter* spp. in 1 gram of soil; the columns designed by the same letter do not differ significantly (P = 0.05); NI – no inoculated; I – inoculated with *Azotobacter* spp. and *Rhizobium* spp.

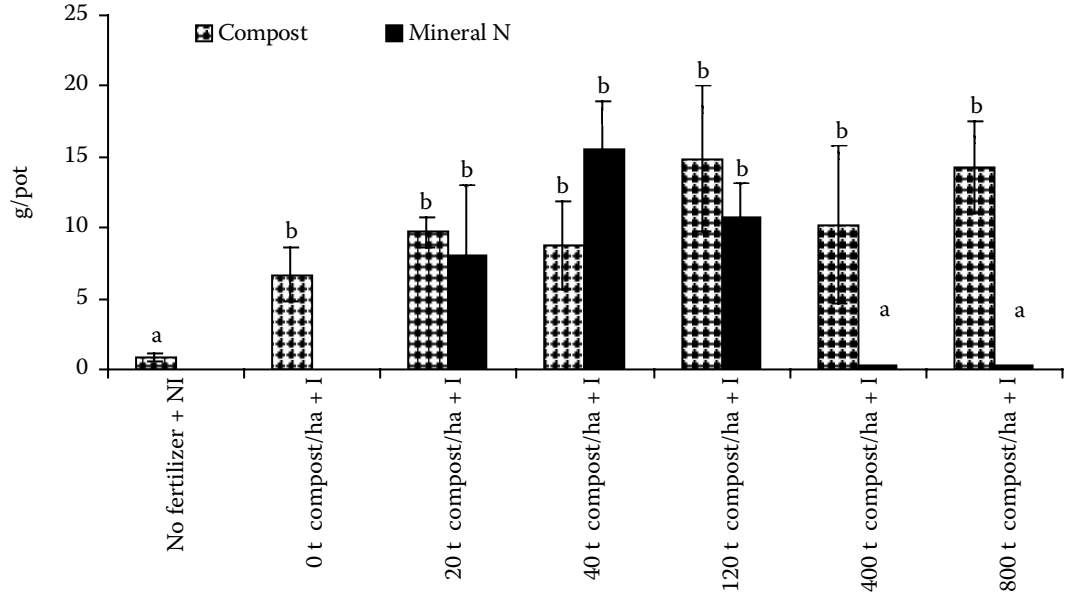

Figure 4. Shoot dry matter of *Medicago sativa*; the columns designed by the same letter do not differ significantly (P = 0.05); NI – no inoculated; I – inoculated with *Azotobacter* spp. and *Rhizobium* spp.

significantly stimulated the growth of *Rhizobium* spp. The numbers of bacteria were significantly lower in variants fertilized by mineral fertilizer in doses that correspond to 120, 400 and 800 t of compost per hectare (in nitrogen content). There was not a significant difference among other variants.

The inoculation significantly stimulated the numbers of *Azotobacter* spp. in the variants fertilized by compost in doses 120, 400 and 800 t/ha. (Figure 3). On the other hand, mineral fertilization in higher doses inhibited the numbers of *Azotobacter* spp. These results completely correspond to published findings by Mikanová *et al.* (1996) that have shown inhibition of the growth of *Azotobacter* spp. by mineral fertilization. The added organic material (in this case compost) increases the number of these bacteria.

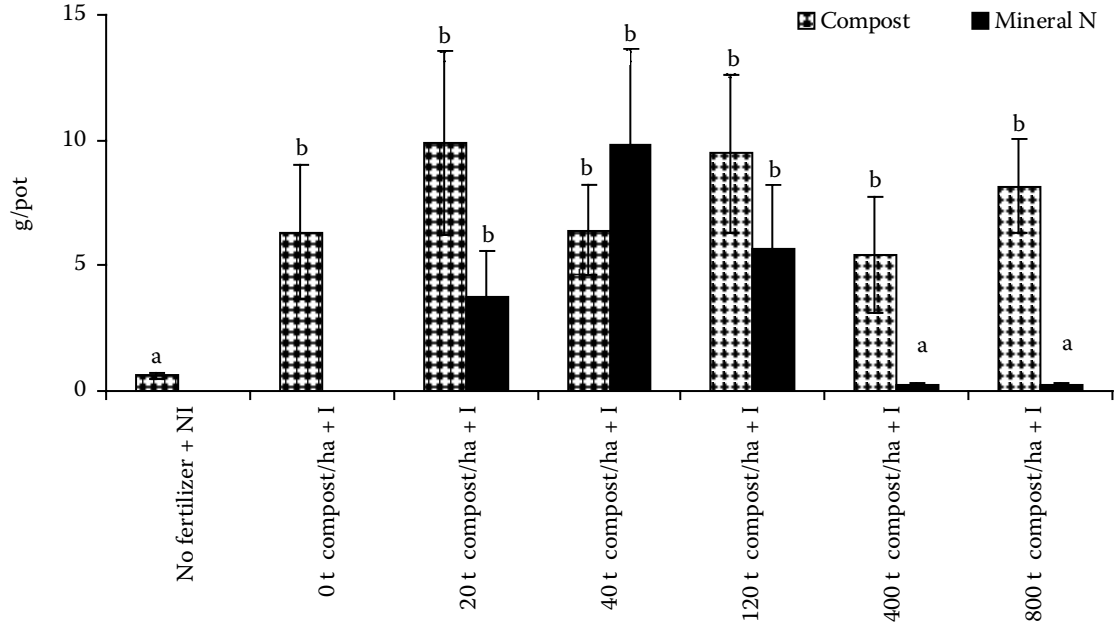

Figure 5. Dry matter of the roots *Medicago sativa*; the columns designed by the same letter do not differ significantly (P = 0.05); NI – no inoculated; I – inoculated with *Azotobacter* spp. and *Rhizobium* spp.

Dry matter of plants increased in the inoculated variants (Figures 4 and 5). The highest doses of mineral fertilizers (corresponding to 400 and 800 t of compost per hectare – in nitrogen content) inhibited germination and growth of plants. There was no statistically significant difference in the plant dry matter and root dry matter among other variants.

CONCLUSIONS

The inoculation significantly increased the nitrogenase activity. The highest nitrogenase activity was found in the variant without fertilization and in the variant with 20 t of compost per hectare. The nitrogenase activity was inhibited by mineral fertilizers in all doses used.

Rhizobium spp. and *Azotobacter* spp. inoculation also significantly increased the dry matter of plants and roots of *Medicago sativa.* Ammonium sulphate in doses corresponding to 400 and 800 t of compost per hectare – in nitrogen content inhibited germination and growth of plants.

The results of the study have proved that compost stimulates the growth of bacteria *Rhizobium* spp. and *Azotobacter* spp. Mineral fertilization in doses corresponding to 120, 400 and 800 t of compost per hectare (in nitrogen content) inhibited the growth of determined bacteria.

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