Restoration of a sandy grassland by the application of various carbon sources promoting the immobilization of soil nitrogen

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Introduction

The rate of succession at disturbed sites is greatly influenced by plant available soil nitrogen (Tilman, 1986; Zink & Allen, 1998). High N availability favours fast growing weeds and invasive plants against native grassland species according to Hueneke and co-workers (1990) as well as McLendon and Redente (1992). The available N in soil is influenced by the rate of microbial N transformation processes (Bolton et al., 1993). Therefore increasing microbial immobilization activity results in a low amount of plant available soil N through the microbial incorporation of nitrogen into biomass. The restoration of disturbed ecosystems or abandoned agricultural fields having elevated soil nitrogen available by organic carbon addition is suggested as a possible technology (McLendon & Redente, 1992). Other nitrogen depleting methods (as topsoil removal or biomass burning) are expensive or may increase the fire hazard. Following the addition of organic carbon substrates, a larger amount of microbial biomass, increased microbial activity and rapid N immobilization were observed both in laboratory (Antal et al., 1988; Gulyás & Füley, 1994) and under field conditions (Wilson & Gerry, 1995; Zink & Allen, 1998; Paschke et al., 2000; Török et al., 2000; Cione et al., 2002; Blumenthal et al., 2003). The most frequently applied carbon sources aiming the promotion of N immobilization for restoration purposes were sucrose (McLendon & Redente, 1992; Paschke et al., 2000; Prober et al., 2005), sawdust (Wilson & Gerry, 1995; Corbin & D’Antonio, 2004; Averett et al., 2004), or sawdust, woodchips and sucrose jointly (Török et al., 2000; Blumenthal et al., 2003; Eschen et al., 2007). Plant biomass production was expected to decrease as a result of reduced available N after carbon addition. The conservation benefit under such conditions can be that invasive plants and weeds are unable to overgrow native species. Several studies established that after carbon treatment there was a decrease in the biomass of some exotic plant species (Reever Morghan & Seastedt, 1999; Blumenthal et al., 2003; Averett et al., 2004). The biomass of native...
plant species increased only in a few cases (McLendon & Redente, 1992; Zink & Allen, 1998; Paschke et al., 2000; Blumenthal et al., 2003). Most studies focused on the aboveground vegetation processes, while – although it would be essential for planning successful restoration – microbial N cycling in soils on abandoned agricultural fields was hardly studied (Klein et al., 1995; Török et al., 2000; Corbin & D’Antonio, 2004; Eschen et al., 2007).

Laboratory and field experiments with carbon addition were carried out between 1998 and 2005 (Török et al., 2000; Elhottová et al., 2002; Szilli-Kovács et al., 2007; Tilston et al., 2009). The first aim was to identify application rates of carbon sources to target nutrient poor soils. In the field, N immobilization and microbial biomass changes were investigated during the 6 years the selected carbon sources were applied. The effects of site exposure and seasonal changes on soil N availability were also investigated.

Material and methods

Experimental sites and soils

The experimental area is located near Fülöpháza (N46°52'; E19°24'), in the Kiskunság National Park (Hungary), on which three abandoned sites were chosen. Cultivation ceased at site M (meadow site) in 1991, while at sites D (depression) and H (hummock) in 1995. Elevation differed among sites, depending on the location within sand dunes. Site M was at the lowest position, site D was situated in a depression between dunes; and site H was at the highest elevation. The detailed description of the vegetation of this area was given in an earlier publication (Török et al., 2000). The soil type is alluvial calcareous sandy soil, poor in humus. At the beginning of the experiment, the 0–20 cm soil layer at the chosen sites had the following characteristics: Site M: Organic C: 0.45%; “total” N: 0.054%, pH: 7.9; Site D: Organic C: 0.36%; “total” N: 0.046%; pH: 7.9; Site H: Organic C: 0.17%; “total” N: 0.021%; pH: 8.1.

Laboratory incubation experiment to study N immobilization induced by organic C addition

Bulk soil samples were collected from the experimental field from the upper 20 cm soil layer for the laboratory experiment. 500g soil was weighted into 600 cm³ plastic cylinders with 7 cm diameter. 2 mg sucrose, cellulose-bead and sawdust were added per g soil. Soil moisture was set to 50% water holding capacity and incubated at 22°C. The initial soil mineral N was about 5 µg·g⁻¹. Soil moisture was checked every other day and supplied if needed. Soil NH₄⁺-N and NO₃⁻-N were measured 1, 3, 8, 15, 29, 42 and 78 days after carbon source addition by steam distillation (Bremner, 1965).

In the next experiment, bulk soil samples were taken on sites M, D and H from
the upper 20 cm layer. 30 g dry weight equivalent soil was weighted into 150 cm³ serum bottles, moistened to 50% water holding capacity and amended with sucrose or sawdust at the following rates: 0, 0.6, 1.2, and 2.4 mg C·g⁻¹ soil as sucrose or sawdust added separately and 2.4 mg sucrose C plus 2.4 mg sawdust C·g⁻¹ soil, in three replicates. They were kept in dark at 20°C for 37 days. At the end of incubation the soils were dried and placed at -20°C until the analysis was performed. Soil NH₄⁺-N and NO₃⁻-N were measured from a 1 M KCl extract using Tecator autoanalyser.

Field experiment with sucrose and sawdust addition

Twelve plots of 10×10 m size were established with six control and six equally treated plots at a site in 1998 year. All treated plots received sucrose only or combined sawdust and sucrose additions periodically resulting altogether 36 plots at the three sites. The sawdust was composed of a mixture of oak (*Quercus* spp.) sawdust and wood-chips from a local sawmill, and the sucrose was commercial beet sugar in various application rates (Table 1). Carbon application rates were adjusted according to the results of the laboratory experiment. The higher humus content required a larger amount of carbon to induce a significant effect. Sucrose was distributed 3-weekly, the sawdust 1 to 3 times between April and October yearly. Total application per year was in relation to the length of the vegetation season. Composite soil samples were taken at 7 points from the 0–20 cm layer of each plot several times during the growing season. Soil moisture was measured from a sub-sample, another one was air-dried for soil chemical analysis and the rest sieved through a 2 mm mesh and stored refrigerated at 4°C for microbial biomass analysis.

**Table 1**
The rate (kg C·ha⁻¹·year⁻¹) and frequency of organic carbon treatments on the three experimental sites

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<td><strong>D site (depression)</strong></td>
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<td>Number of treatments per year (sucrose)</td>
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<td>Number of treatments per year (sawdust)</td>
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Analytical methods

Concentrations of extractable N (as both NH$_4^+$-N and NO$_3^-$-N) were measured using a Tecator autoanalyzer for the analysis of 1 M KCl extractions of air-dried samples. Soil microbial biomass N was estimated according to Brookes et al. (1985).

Soil available N was assessed by in situ ion exchanger resin bag or IER-bag method (Binkley & Matson, 1983): 10g mixed-bed ion-exchanger resin (MB-3, Merck®) was sewed into nylon mesh bags, with a 50 cm long coloured string attached to them for easy retrieval. The prepared bags were placed into the soil at a 5–8 cm depth in the centre of each plot in two repetitions and were changed 1.5 monthly during the vegetation season, when sucrose was applied. The collected bags were dried and cleaned in the laboratory, then were extracted with 1 M KCl solution and NH$_4^+$-N and NO$_3^-$-N were measured by steam distillation.

Statistical analyses

In the first laboratory experiment the t-test was used to compare control and C-amended soils at each sampling. In the second laboratory experiment the soil, the sucrose and sawdust additions were the main factors using ANOVA. In the field experiment the effect of treatments was evaluated by mixed ANOVA, the carbon treatment and the year of application were the two main factors and the seasons within a given year were considered as sub-factor. The three sites were evaluated separately, as they differed in the rate of carbon source amendment.

Results

Laboratory incubation experiment to study N immobilization induced by organic C addition

In the first incubation experiment, in which sucrose, cellulose bead and sawdust were mixed with soil in 2 mg·g$^{-1}$ concentration, a significant net N mineralization was observed in the control soil (reaching 15 µg·g$^{-1}$ on the 15th day of incubation), while the soil mineral N content of carbon source treated soil remained 2 to 3 µg·g$^{-1}$ for a long time (Fig. 1). It was shown that soil mineral N could be kept as low as 3 µg·g$^{-1}$ soil for 2–3 months if a combination of sucrose, cellulose-bead and sawdust was applied under laboratory conditions.

In the second incubation experiment, in which carbon sources were applied at similar rates as in the field sites, the soil samples were taken directly from sites M, D and H. Soil nitrate N decreased in carbon amended soils with the exception of the 0.6 mg sucrose C·g$^{-1}$ soil treatment for sites M and D and the 1.2 mg sucrose C·g$^{-1}$ soil treatment for site M after 37 days of incubation. To obtain a significant net N immobilization as compared to the control, in the case of sucrose amendment the lowest carbon rate for H, the mid-rate for D and the highest rate for M was effective (Fig. 2). All sawdust treatment rates increased the net N immobilization of all soils.
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Fig. 1
Extractable mineral N content of soil amended with sucrose, cellulose-bead and sawdust at 2–2 mg g⁻¹ soil rate during 78 days incubation at 22°C. Each value is the mean of 3 replicates and the bars represent +/- standard deviations. Horizontal axis: number of days. Vertical axis: NH₄⁺NO₃-N, μg g⁻¹ soil

The 2.4 mg C·g⁻¹ soil combined sucrose and sawdust treatment resulted in the highest N immobilization rate at the end of incubation. There was no further increase in net N immobilization with the application of sucrose and sawdust in higher doses (data not shown).
Microbial biomass N

In the field experiment there was a significant increase in soil microbial biomass N in carbon source treatments at sites $M$ and $D$, while the increase was only slight at site $H$. Out of the 11 sampling dates microbial biomass N was higher in carbon source treated plots in many occasions as compared to the control (Fig. 3). The sampling year also had a significant effect on the quantity of microbial biomass N, with the exception of site $H$. The values significantly differed in 2001 from the other two years at sites $M$ and $D$. The seasonal effects on microbial biomass N were non-significant at two sites, while they were even less in April 2001 at site $M$. No significant difference was found in microbial biomass N between May and September samplings in 2000 and 2002 at sites $M$ and $D$. Comparing the three sites, soil microbial biomass N consequently differed in the order of $M > D > H$, which reflects the soil humus content.

Soil ammonium and nitrate-N

Soil extractable mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) was reduced significantly by the field application of sucrose and sawdust at all three sites. On 8 sampling dates out of 11, a significant decrease in soil mineral N was observed at least at one site (Fig. 4).
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Fig. 4
Changes in soil mineral N (NH$_4^+$-N and NO$_3^-$-N) in the years 2000 to 2003 in the control and organic C treated plots of the three experimental sites (M: meadow; D: depression; H: hummock). Asterisk with a letter shows if the soil mineral N at treated plots significantly differ from the control value. Horizontal axis: date of sampling, (month, day, year).
Vertical axis: Soil mineral N, µg N·g$^{-1}$ soil

The soil NH$_4^+$-N content was relatively low and ranged between 0.8 and 8.2 mg·kg$^{-1}$ soil. Organic C addition reduced soil NH$_4^+$-N significantly only at site M, while a slight decrease was observed at sites D and H. The year and season effects were significant at all sites. The soil NH$_4^+$-N content was the lowest in May, as compared to April and September samplings. The soil NO$_3^-$-N content was generally lower than the NH$_4^+$-N content and ranged between 0 and 5.7 mg·kg$^{-1}$ soil. The soil NO$_3^-$-N content was significantly reduced by organic C addition at sites M and D. The year and season effects were significant on soil NO$_3^-$-N at all sites. The highest soil NO$_3^-$-N was measured in September, while it did not differ significantly between April and May on all sites.

Soil N availability based on IER-bags
The NO$_3^-$-N extracted from resin bags significantly differed by treatments, years and seasons. Especially the three sites and seasons showed obvious differences. The difference in N availability between the control and organic C amended plots varied from site to site, with the highest value observed at site M, followed by site D and then site H (Fig. 5). The NH$_4^+$-N extracted from resin bags was reduced in treated plots only in a few cases.
Fig. 5

NO₃⁻-N content adsorbed by the ion exchange resin during three consecutive vegetation periods at the meadow (M), depression (D), and hummock (H) sites from control and organic C treated plots (A: 2001, B: 2002; C: 2003)
Discussion

The laboratory incubation experiment clearly demonstrated that the application of sucrose and sawdust at a 2 mg·g⁻¹ soil rate may affect and lower the available (i.e. ammonium and nitrate) nitrogen content in soil for a long time, up to 80 days. It was also shown that net N immobilization differed in soils originating from various sites. Therefore, the rate of carbon source application in field was adjusted to fit the particular site the best, on the basis of the laboratory incubation results.

In the first year of the field carbon source treatment in 1998, microbial biomass increased significantly at site D, while soil NO₃⁻-N decreased at sites D and H. The frequency and doses of sucrose and sawdust application was increased in the following years and varied among sites. This resulted in significant microbial biomass increase at sites M and D, and only once at site H, although microbial biomass estimation by other methods (fumigation-incubation and substrate induced respiration) showed significant increase at all sites due to the treatments (SZILLI-KOVÁCS & TÖRÖK, 2005).

Microbial biomass in soil reached the maximum value after 2 to 4 days of glucose addition and 12 to 60 days later returned to the initial level, while the glucose was fully consumed within 20 days (NANNIPIERI et al., 1978; WU et al., 1993). Under field conditions, in which soil moisture and temperature are far from being optimal for microbes, it can take longer. Microbial biomass N doubled (CORBIN & D’ANTONIO, 2004) when sawdust was added to the field in approximately 10-times higher dose than in the presented experiment. Microbial biomass increase was also observed in field after applying sucrose and sawdust together in England and Switzerland at four sites (ESCHEN et al., 2007). In addition, a significant increase in the fungi to bacteria ratio was found at one site, which was observable even a year after the treatment (ESCHEN et al., 2007). That reflects not only biomass, but structural changes in the soil microbial community as well. Fungal biomass increase was also observed even 2 years after oat straw and pine bark addition (ZINK & ALLEN, 1998). In the present study the microbial biomass C to N ratio was higher only at site H in carbon amended plots, resulting probably from the shift of fungal to bacterial biomass. The complex organic materials of wood can be decomposed primarily by saprotrophic fungi. The slow decomposition, however, results only a small increment in fungal biomass. Actinobacteria are also known to decompose woody organic matter and a high quantity of these could be observed in the sandy soil of our sites (ELHOTTOVA et al., 2002). Decrease in soil NH₄⁺-N and NO₃⁻-N can obviously be attributed to treatments, because after the cessation of treatment, soil NH₄⁺-N and NO₃⁻-N contents increased again. This corresponds with the results of ESCHEN and co-workers (2007). In accordance with PASCHKE et al. (2000) and CIONE et al. (2002), the presented study also revealed that the effect of carbon addition on N immobilization can be indicated better by NO₃⁻-N extracted from resin than by NO₃⁻-N extracted from soil, as the NO₃⁻-N adsorbed by resin showed the cumulative effect during the whole exposition period.

We found that sucrose and sawdust application in field resulted in a significant increase in soil microbial biomass. The repeated carbon source addition helped to
sustain enhanced soil microbial biomass. Soil available N, especially NO₃⁻-N, decreased as a result of sucrose and sawdust treatments. Efficiency of carbon source treatment in decreasing soil available N varied from site to site and also by seasons, which reflects the influence of abiotic factors (e.g. precipitation) on N immobilization and mineralization processes.

**Summary**

Various carbon sources were applied to promote the microbial immobilization of soil nitrogen in order to facilitate the regeneration of native vegetation on abandoned arable land with sandy soil. The aim was to investigate whether carbon addition affected the microbial biomass and available nitrogen content of the soil.

The experimental area was located on an abandoned farm near Fülöpháza in the Kiskunság National Park, where three sites (H = Hummock, D = Depression, M = Meadow) were selected. Sucrose and a mixture of hardwood sawdust plus woodchips were applied several times during the growing season for 6 years. Soil available N, especially NO₃⁻-N was significantly reduced at all sites, based on both soil analysis and on the results of ion-exchange resin tests. The quantity of microbial biomass was significantly greater in carbon treated plots compared to the control at sites M and D in many times but only once at site H. The efficiency of carbon source treatment varied in different years and seasons. The microbial biomass was generally smaller in April than in May or September, while the soil NO₃⁻-N content was the highest in September. The combined application of sucrose and sawdust resulted in a significant reduction in the available N content of the soil. This decrease in N availability can promote the growth of natural grassland species at the expense of weeds with high N demands and accelerate the restoration process.

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**Key words:** N-immobilization, microbial biomass, sucrose, sawdust, carbon addition

**References**


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