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MEADOW PLANT LITTER SPECIES DIVERSITY – IMPACT ON ORGANIC MATTER ACCUMULATION

ABSTRACT

We evaluated the effect of plant litter species diversity on the on humus accumulation in the underlying substratum and on soil microbial communities composition in experimental field study.. Sandy loam (sand mixed with loam) mesocosms of were examined over the course of 2.5 years. Plots contained simplified substratum –. Five litters composed of meadow plants; both grasses and herbs (weed species) were used. Litters were composed of single species (I - *Dactylis glomerata*, II - *Festuca rubra* and III - *Trifolium pratense*) or were species mixtures IV (mixture of 3 species I, II and III); V (mixture of 12 species - IV and 9 other grasses and meadow weeds). Results baased mainly on the last year of the experiment show that most of the differences among treatments found in the substratum below the litter cover resulted from the litter diversity. Soil respiration, microbial biomass and numbers of fungi and bacteria did not differentiate between mono- and multispecies treatments. Despite of that the soil respiration and algal biomass were significantly correlated during the study period. The biomass of algae as well as carbon and nitrogen increases in the substratum, depended on the litter diversity and were higher when litter composition was more complicated. In general, our results suggest that algae may participate in the process of humus formation. .

Key words: algae, microorganisms, carbon, nitrogen, humus, litter

INTRODUCTION

Compared to native plant communities drastic reduction of plant species richness are commonly observed in agricultural ecosystems (Giller *et al.* 1997, Chapin *et al.* 2000, Hooper *et al.* 2005). The number of weedy plants in arable lands, over the past decades dramatically declined (Marshall *et al.* 2003, Boatman *et al.* 2007). While this trend may be good for crop productivity, the impact

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of decreasing diversity both taxonomic and functional on soil processes is largely unknown (Ryszkowski *et al.* 1990, Kajak, Wasilewska 1997, Wardle *et al.* 1999, Spehn *et al.* 2005). Studies of cereal fields have demonstrated negative effects of monocultures on the content and quality of humus (Ryszkowski *et al.* 1990, Łoginow *et al.* 1990, Gołębiowska *et al.* 1990). Intensification of agriculture (large doses of mineral fertilisers, herbicides and pesticides) often lead to losses of organic matter, primarily humus (Lal 1999, Freibauer *et al.* 2004). Humus plays an important role in the ecosystem functioning. It stores many elements and improves soil structure and biological activity.

Our previous research has led to a general understanding of the relationship between carbon sequestration and food web complexity allowed for concluding that increased carbon sequestration proceeded more intensively in ecosystems with diverse vegetation and more complex food webs than in those with simplified species composition (Dekker et al 2005, Kajak *et al.* 1997). Similarly, Ponge (2003) suggested, that humus plays a central role in the functional diversity of terrestrial ecosystems.

Very little is currently known about the importance of decomposing species diversity. Changes in microbial and algal diversity are particularly neglected due difficulties in determining of species composition in these groups. Results of some investigations suggest, however, that microbial diversity enhanced soil functioning and resilience (Pearl, Pinckney 1996, Van den Heijden 1998, Liebich *et al.* 2006).

In this paper we present the results of the experiment on the effect of plant litter species richness on the relationship between the impoverishment of communities and humus accumulation in soil. Our primary objective was to assess the hypothesis that the litter composed of diverse meadow plant species including weedy ones enhances the activity of decomposers and accumulation of humus in soil.

METHODS

The study was conducted in a permanent meadow (of the type Arrhenathereta-lia) situated in the buffer zone of the Kampinos National Park (middle Poland) (Szanser 2000).

The experimental meadow of an area of 190 x 10 m had 110 microplots (0.5 x 0.5m area and 0.15 m depth).Five litter treatments in 22 plot replicates according to RCB design were applied (Pearce 1983). Eight litter containers per plot were placed. Litter was obtained from meadow plants, both grasses and weeds cut in August 2001 which had to simulate the input of decaying plants to soil. The same amount of litter (9 g dry wt.) irrespective of the number of plant species was exposed in modified litter containers (Ilieva-Makulec et al 2006). Experimental plots were filled with sand mixed with loam to the depth of 15 cm.

Such type of simplified soil had to increase sorption capacity of substratum and to decrease outwashing of humic substances (Kajak *et al.* 2000). Sand in plots was separated from surrounding soil by a foil which had to prevent from roots growing into experimental substratum.

Litter of the following species composition was applied: I – The cocksfoot – *Dactylis glomerata* (C:N ratio = 20.79), II – The red fescue – *Festuca rubra* (C:N ratio = 38.42), III – The red clover – *Trifolium pratense* (C:N ratio = 18.24), IV – Mixture of the three plant species from treatments I, II and III applied in equal proportions of the (C:N ratio = 20.28), V – Mixture of 12 plant species (combined litter of I – III + 9 other species; (C:N ratio = 19.97). The last treatments was composed of grasses: the brome grass – *Bromus inermis*, the meadow foxtail – *Alopecurus pratensis*, the perennial ryegrass – *Lolium perenne*, the oat grass – *Arrhenatherum elatius*, the cocksfoot – *Dactylis glomerata*, the red fescue – *Festuca rubra* and herbs (weedy species): the small plantain – *Plantago lanceolata*, the common chicory – *Cichorium intybus*, the red clover – *Trifolium pratense*, the milfoil – *Achillea millefolium*, the carrot – *Daucus carota*, the common silverweed – *Potentilla anserin*a.

Composition of mixtures was selected in a way that the basic differentiating parameter was the number of plant species and not chemical composition. Some differences of the litter quality could not be avoided but mixtures had C:N ratios intermediate between those of single-species litters. Treatments in plots were arranged according to random blocks.

Sampling and analyses

The experiment started on 24 – 25 March 2002. Samples were taken on 25 June 2002, 27 September 2002, 11 May 2004, 9 September 2004 i.e. 3, 6, 26 and 30 months since litter exposure. The number of samples and sampling frequency is presented in Table 1.

The analyses involved an assessment of the content of organic carbon and nitrogen content in underlying substratum. The number of bacteria and fungi, and release of $CO_{2,}$, microbial biomass as well biomass and production of soil algae were determined.

Sampled plots were randomly chosen on every sampling occasion. Organic carbon was analysed with Shimadzu TOC 5000A analyser. The content of nitrogen was analysed with the Kjeldahl method using Kjeltec analyser made by Tecator. All the methods concerning soil organic matter are described in Kisiel (2005). Soil microbial activity assessed as a basal respiration using absorption method (in 2002) and infrared method (in 2004) with IRGA apparatus model ADC 225 MK3 (Wojewoda and Russel 2003). The infrared method was applied for estimating microbial biomass assessed by SIR - substrate (glucose) induced

respiration (Anderson and Domsch 1978). Plate method (Bunt and Rovira 1955, Martin 1955) was used to estimate the share of functional groups in the microbial community.

Algal biomass was estimated according to the methods of Wood (1975) and Sieminiak (1996) with the UV/VIS spectrophotometer model V-350 JASCO. Algal production was determined from changes in the biomass, duration of life cycles, growth rates and reproduction rates of soil algae (Petrusewicz, Macfadyen 1970, Sieminiak 1996). Samples for estimating the chemical and microbiological parameters were taken from the substratum to the depth of 5 cm with soil corers of area 100 cm^2.

Table 1

Differences between treatments in underlying substratum during the third year of the experiment. On every sampling occasion 6 samples per treatment were taken and pooled to 3 (different than 6 number is denoted). Differences between treatments were assessed using the multifactorial ANOVA. The other tests applied are denoted. Denotations of treatments: I – *Dactylis glomerata*, II – *Festuca rubra*, III – *Trifolium pratense*, IV – mixture of plants in treatments I, II and III, V – mixture of 12 meadow plants species plants (those ones as in treatment IV + 9 other species)

Parameter	Applied test	P	Significant differences between treatments
C org. increment[1,2]	U = 9.5, Z = -2.087	= 0.036	V, IV>I, II, III
N_{total} increment[1]	$F_{(1.14)}$=17.44	<0.002	V, IV>I, II, III
Bacteria[3]	$F_{(1.49)}$=0.04	<0.85	n.s.
Fungi[3]	$F_{(1.49)}$=0.24	<0.62	n.s.
Respiration[4]	$F_{(1.29)}$=0.09	<0.77	n.s.
Microbial biomass[4]	$F_{(1.29)}$=0.90	<0.35	n.s.
Biomass of algae	$F_{(1.29)}$=9.81	<0.005	V>I, II, III, IV
Production of algae[5]	$F_{(1.8)}$=11.08	<0.01	V, IV>I, II, III

1 – between 26 and 30 month of the experiment (2 samplings),
2 – U-Mann-Whitney test,
3 – 2 samplings taken as denoted in 1 with 5 samples per treatment,
4 - respiration and microbial biomass assessed by infrared method method (2 samplings taken as denoted in 1)
5 - algal production for entire time of the experiment

Statistical processing of results (multifactorial ANOVA) was performed with the Statistica 6.1 software. Kendall test, U-Mann-Whitney test and one way ANOVA were applied for testing the relation between algal production and nitrogen increase and effect of treatments on carbon and nitrogen content jn soil respectively.

In the paper we present mainly results from the end of the experiment and when is needed data from the whole period are presented.

RESULTS

The humus and nitrogen increases in the substratum during the last year of the experiment were significantly higher under the litter mixtures than under single species treatments (Table 1). Increase of carbon under single

species comprised 64% of value for mixtures, respectively 29.04 (4.14) and 45.54 (7.67) mg × 100 g^{-1}. Nitrogen increase under single species litters was only 12% of the value for mixture treatments, respectively 0.30 (0.13) and 2.55 (0.64) mg × 100 g^{-1}. Mono- and multispecies treatments did not differ from each other in term of the number of bacteria and fungi in the underlying substrata in that period (Table 1). At the end of the experiment no significant differences between experimental treatments were found in basal respiration (Table 1) or in microbial biomass (Table 1). The algal biomass was significantly correlated with soil respiration in every four consecutive sampling times ($r = 0.92$, $P < 0.05$, $r = 0.85$, $P < 0.05$ in 2002 and $r = 0.35$, $P < 0.05$, $r = 0.82$, $P < 0.05$ in 2004).

The biomass of algae was very sensitive to the species richness of litter cover. During the last year of the experiment the highest algal biomass was found under the most diverse litter comparing to other treatments (Table. 1). Difference between the diverse litter treatment and the other treatments was highly significant (Table 1).

The algal production under the litter composed of single plant species, was higher than under the species mixtures. The differences between the single species and mixtures were significant (Table 1). We also found a positive correlation between the algal production and the total N as well as the organic C increases in the substratum during the last year of the experiment (Kendall test, $P = 0.014$, $T = 1.000$, $Z = 2.449$, $n = 5$ and $y = 0.1961x - 1.5301$, $R2 = 0.9785$, $P < 0.002$, $F = 136.78$, $n = 5$ respectively). Algal biomass was significantly correlated with soil respiration in every four consecutive sampling times ($r = 0.92$, $P < 0.05$, $r = 0.85$, $P < 0.05$ in 2002 and $r = 0.35$, $P < 0.05$, $r = 0.82$, $P < 0.05$ in 2004).

DISCUSSION

The environmental conditions did not differ among experimental treatments (Szanser *et al.* in prep).

The preliminary results of our experiment support the hypothesis that the plant litter diversity is an important factor for humus formation in soil. The organic carbon increases were indeed significantly higher below the litter mixtures, than under that composed of a single plant species. It should be stressed that changes in carbon and nitrogen increases under the litters, between 26 and 30 months, were not related to litter mass loss. The slowest litter mass loss was observed for Fescue and the highest for clover and the most diverse litter in the first year of the experiment (3 and 6 months after exposure). Later data (26 and 30 months) showed no differences between treatments (Szanser *et al.* (in prep.). Weeds comprised half of the initial mass in the most diverse treatments. Weeds diversity is known to be

important for invertebrate and vertebrate animals functioning (Marshall *et al.* 2003, Boatman *et al.* 2007). Our data also indicate its importance for soil ecosystems.

It is not known too much about soil algae role in humus formation. Our results suggest that these biota might be underestimated as a source of newly formed humus comparing to other plant derived materials entering the soil system. .

The analysis restricted to the litter but neglecting underlying substratum (Schädler, Brandl 2005, Wardle *et al.* 2006, Jonsson, Wardle 2008) narrows the possibility to find the system response. In our experiment the effect of the litter species richness was much more evident in the substratum than in the litter itself, while its quality (C/N) quite often decided on the differences found inside the litter (Ilieva-Makulec et al 2006, Szanser *et al.* in preparation).

In conclusion, our results suggest that the plant litter diversity is important for soil algae functioning and these organisms might be involved in humus formation.

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