^{15}Nitrogen uptake from shallow- versus deep-rooted plants in multi-species and monoculture grassland

Karin Pirhofer-Walzl[1], Henning Høgh-Jensen[2], Jim Rasmussen[3], Jesper Rasmussen[1], Karen Søegaard[3] , Jørgen Eriksen[3]

[1]Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Højbakkegaard Allé 9, 2630 Taastrup, Denmark
[2]National Environmental Research Institute, Department of Policy Analysis, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark
[3]Department of Agroecology and Environment, Research Centre Foulum, Aarhus University, Blichers Allé, 8830 Tjele, Denmark

Abstract

Only few studies have explored the importance of functional diversity in temperate agricultural grasslands in relation to active rooting depth. This study investigates the consequence of adding deep-rooted plants to grass-clover mixtures in terms of nitrogen uptake efficiency from deep soil layers. The objective was to compare the active rooting depth of the shallow-rooted grassland species *Lolium perenne* and *Trifolium repens;* and the deep-rooted species *Cichorium intybus* and *Medicago sativa* in monocultures and mixtures. We hypothesized that adding deep-rooted plant species to a plant community with shallow-rooted species increases the active rooting depth partly through competition. A ^{15}N tracer study was carried out with ^{15}N enriched ammoniumsulphate placed at three different soil depths (40, 80 and 120 cm). To recover ^{15}N, above-ground plant biomass was harvested after 10 days. We described the decline of ^{15}N uptake with depth by using an exponential decay function. The studied plant communities showed the same relative decline in ^{15}N uptake by increasing soil depths, but different capacities in total ^{15}N uptake. Monoculture *L. perenne* foraged less ^{15}N in all depths compared to the other four plant communities. The relative ^{15}N uptake of individual plant species grown in mixture decreased stronger with depth than in monoculture. Both findings rejected our hypothesis.

Keywords: deep-rooted, shallow-rooted, ^{15}N uptake, multi-species, grassland

Introduction

Perennial grassland systems are widely managed as grass monocultures or two-species mixtures in temperate climates, where especially grass monocultures require high nitrogen inputs. A number of studies examined grassland diversity in terms of nitrogen supply from legumes. Less examined is the plant diversity in perennial grassland based on different root depths. Multi-species grasslands with plants differing in the rooting system could increase the nitrogen use efficiency of the whole plant community by complementary nutrient uptake. In this field experiment we investigated the importance of functional diversity in relation to active rooting depth by measuring the nitrogen uptake in different depths (40, 80 and 120 cm). The active rooting depth of the shallow-rooted *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) and the deep-rooted species *Cichorium intybus* (chicory) and *Medicago sativa* (lucerne) was compared in monocultures and mixtures. We hypothesized that the active rooting depth could be increased by adding deep-rooted species to a plant community with shallow-rooted species and that competition increases the active rooting depth in multi-species mixtures.

Materials and methods

Five grassland plant communities were grown in four replicate plots at the Research Farm of Copenhagen University in Denmark from September 2007 to October 2009 (Table 1). The plots were cut four times in both growing seasons and dry matter yield and botanical composition were determined. On the 8^{th} of June 2009, 25 days after the first cut, ammoniumsulphate, enriched with the heavy nitrogen isotope ^{15}N (99.5 atom %), was placed in 40, 80 and 120 cm depth (156.25mg $^{15}N/m^2$). The ^{15}N tracer was expected to be taken up by the plants and therefore measureable as the ^{15}N uptake (mg^{15}N uptake/m^2) in the above-ground plant biomass. Regression analysis was used to analyze how N uptake was influenced by depth in different plant communities and how competition influenced the relative decline of N uptake by depth.

Results and discussion

Nitrogen input from atmospheric fixation can partly explain that plant communities including legumes, especially *M. sativa* and the four-species mixture, were highly productive, while monoculture *L. perenne* showed consistently low yields (Table 1). However, all communities showed relative high productivity on the 2^{nd} cut in 2009, shortly after the ^{15}N placement study was conducted. The botanical composition was similar for individual plant species in the mixtures on the 1^{st} and 2^{nd} cut 2009, respectively. However, *L. perenne* showed the lowest and *C. intybus* the highest proportion of dry matter in the four-species mixture (Table 1).

Table 1. Plant community, mean yield and mean botanical composition in 2009. Values are means ± SE. Different lowercase letters show significant difference determined by Tukey HSD test at P=0.05.

Plant community	**Yield (kg dry matter/ha)**				**Bot. composition (% of dry matter)**			
Cut	**1^{st}**	**2^{nd}**	**3^{rd}**	**4^{th}**	**1^{st}**	**2^{nd}**	**3^{rd}**	**4^{th}**
L. perenne	600^{a}	1038^{a}	197^{a}	103^{a}				
C. intybus	1975^{bd}	2810^{b}	1397^{bd}	1231^{b}				
M. sativa	3998^{c}	3510^{c}	2960^{c}	2510^{c}				
T. repens	1762^{d}	3452^{c}	862^{ab}	741^{b}	47.9	53.8	68.1	57.9
L. perenne					52.1	46.2	31.9	42.1
M. sativa					22.1	23.0	53.6	61.1
C. intybus	2367^{bd}	3575^{c}	2121^{cd}	2050^{c}	33.0	38.5	34.4	30.3
T. repens					23.1	23.9	8.6	5.6
L. perenne					21.8	14.6	3.5	3.1

All plant communities (Table 1) showed the same relative decline in ^{15}N uptake by increasing soil depths ($p=0.353$; Fig 1a) and there was no significant difference in total ^{15}N uptake among plant communities. However, monoculture *L. perenne* showed lower ^{15}N uptake than the rest. Thus, there was no indication of increased active rooting depth in plant communities including deep-rooted species. The observed means of ^{15}N uptake in the four-species and two-species plant community appeared to be higher in 40 cm depth than in the monocultures, but the difference was not significant ($p>0.05$). The two legumes, *M. sativa* and *T. repens*, could have influenced the N-cycle in the plant communities, enhancing the growth of both the legumes and non-legumes (Rasmussen et al. 2007). Felten et al. (2009) conclude from a field experiment on natural grassland that a high soil N pool in communities including legumes can make it unnecessary for deep-rooted plant species to explore N resources in deeper soil layers. Active rooting depth can also change throughout the growing season, as Veresoglou and Fitter (1984) showed on five co-existing grasses. The slopes of the exponential decay functions were used to compare the ^{15}N uptake of individual plant species grown in monoculture or in a four-species mixtures. The active rooting depth decreased relatively stronger when plant species were grown in mixtures ($p<0.0001$, Fig 1b).

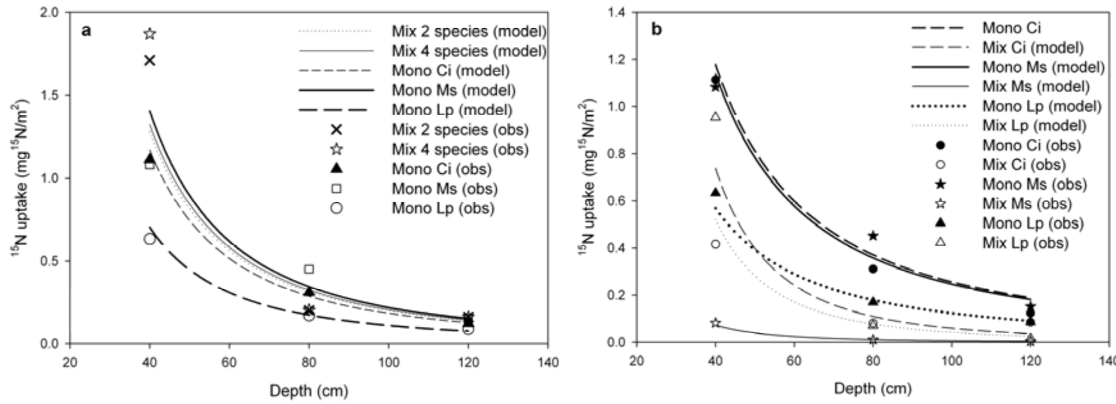

Fig 1. Observed values and predicted curves from regression analysis describing the ^{15}N uptake in above-ground plant tissue 10 days after ^{15}N placement relative to a) depth and plant communities and b) depth and whether plant species were grown in mono- or mixed plant communities. Mono= Monoculture, Mix= Mixture, Lp=*L. perenne*, Ms= *M. sativa*, Ci=*C. intybus*, obs= observed values and model= predicted curve.

M. sativa can obtain nitrogen from two pools: the atmospheric nitrogen pool and the soil nitrogen pool. When competing in the mixture, the deep-rooted *M. sativa* could forage nitrogen in deep soil layers, but instead, our finding indicates that *M. sativa* invests in atmospheric nitrogen fixation. Furthermore, the legumes in the mixtures could have donated atmospheric nitrogen to neighbouring plants (Rasmussen et al. 2007) and thus, measurements of ^{15}N uptake in plant communities with legumes could have been biased.

Conclusion

It was not possible to show an increase in root activity by depth by adding deep-rooted species (*M. sativa* and *C. intybus*) to a plant community with shallow-rooted species (*T. repens* and *L. perenne*). And there was no increase in active rooting depth when plant species were grown in competitive multi-species mixtures. The study could not support that the deep-rooted species, *M. sativa* and *C. intybus* are relatively more efficient in N uptake in deep soil layers and that they add a new 'deepness' function to communities with shallow roots.

Acknowledgments

We would like to thank Anders Nørgaard, Morten Lykkebo, Britta Garly Henriksen and Allan Esben Hansen for technical and practical support and Anja Hecht-Ivø for ^{15}N analysis. The International Centre for Research in Organic Food Systems (ICROFS) funded the project.

References

Felten v S., Hector A., Buchmann N., Niklaus P.A., Schmid B. and Scherer-Lorenzen M. (2009) Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. Ecology, 90, 5, 1389–1399.

Rasmussen J., Eriksen J., Jensen E. S., Esbensen K. H. and Høgh-Jensen H. (2007) In situ carbon and nitrogen dynamics in ryegrass-clover mixtures: Transfers, deposition and leaching. Soil Biology & Biochemistry 39, 804-815.

Veresoglou D.S. and Fitter H. (1984) Spatial and temporal pattern of growth and nutrient uptake of five co-existing grasses. Journal of Ecology, 72, 259-272.