ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 22 Number 5, 2011

THE AVAILABILITY OF MINERAL NITROGEN IN MEDITERRANEAN OPEN STEPPE DOMINATED BY *STIPA TENACISSIMA* L.

I. Novosádová, J. Záhora, J. D. R. Sinoga

Received: April 12, 2011

Abstract

NOVOSÁDOVÁ, I., ZÁHORA, J., RUIZ-SINOGA, J. D.: *The availability of mineral nitrogen in Mediterranean open steppe dominated by Stipa tenacissima L.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 5, pp. 187–192

The area of interest is located in the Sierra de los Filabres in semi-arid steppe of the province of Almeria in Spain. The amount of water in the soil is a limiting factor and its availability affects the structure and species composition of ecosystem. On the other hand, the type of vegetation affects the water loss via evapotranspiration and thus the soil microclimate. It has a great influence on the growth and activity of soil microbial communities and hence the dynamics of decomposition of organic matter and nutrient availability. The aim of this study was to assess the intensity of microbial transformations of soil organic nitrogen and describe changes in the content of nitrogen mineral forms at different depths in the semi-arid climate soil in the Mediterranean region. Availability and movement of nitrogen was monitored by capturing the mineral nitrogen into the structures of ion exchange resin applied to the soil in three different variants (control variant, a variant with the addition of cellulose, and the variant with the addition of raw silk). Ion exchange resins have been installed into soil profile in 2008, 2009, and 2010. After the *in situ* exposure the ion exchange resins were removed from the soil profile and the quantity of captured mineral N was determined by distillation titration method. The availability of ammonia-nitrogen was significantly affected by the addition of different substrates mainly by the additions of the raw silk, where the availability was regularly the highest. However, the availability of ammonia-nitrogen form was generally higher than the availability of nitrate form.

Mediterranean soil, mineral nitrogen, microbial transformations, ion exchange resin

The microbial activity plays an important and key role in the soil processes; first of all in the transformation of different organic compounds entering the soil milieu, in the formation of persistent soil organic matter and in the sustainable releasing of the nutrients. Also soil fauna may substantially affect the living conditions of microflora and thus indirectly affect microbial activity (Frouz and Nováková, 2005). The soil microbial biomass is a labile pool of organic matter and comprises 1–3% of total soil organic matter. The soil microbial biomass acts as a source and sinks of the plant nutrient and regulates the functioning of the soil system (Kaur *et al.*, 2000). These source-sink dynamics depend on complex interactions between climate, topography, vegetation and soil surface properties (Maestre, 2006). Plant cover through its effects on the quantity and quality of organic matter inputs influences the levels of soil microbial biomass (Kaur *et al.*, 2000). The nutrient release processes have a fundamental role in ecosystem functioning, particularly in Mediterranean areas, where nutrient availability, mainly nitrogen, represents a limiting factor (Rutigliano *et al.*, 2009). However, in soil with less nitrogen the nutrient cycle is subject of strict biological control. Plant litter is attacked by decomposers, and almost all the nitrogen it releases is immediately immobilized by microorganisms. In Mediterranean areas the nutrient availability is also closely linked to water availability in the soil.

This strong link between N availability and water use efficiency makes particularly important the understanding of factors affecting soil N availability in Mediterranean ecosystem in view of the future predicted increasing drought in this area (Rutigliano *et al.*, 2009).

Open steppes dominated by tussock grass *Stipa tenacissima* constitute one of the major vegetation types in the driest areas of the Mediterranean basin. *Stipa tenacissima* steppes are good model systems in arid land ecology, as the vegetation patterns and the processes governing their functioning resemble those described in arid and semiarid regions throughout the world (Maestre, 2006). The aim of this study was to assess the intensity of microbial transformations of soil organic nitrogen and describe changes in the content of nitrogen mineral forms in different depths of the Mediterranean soil which is influenced by tufts of grass *Stipa tenacissima*.

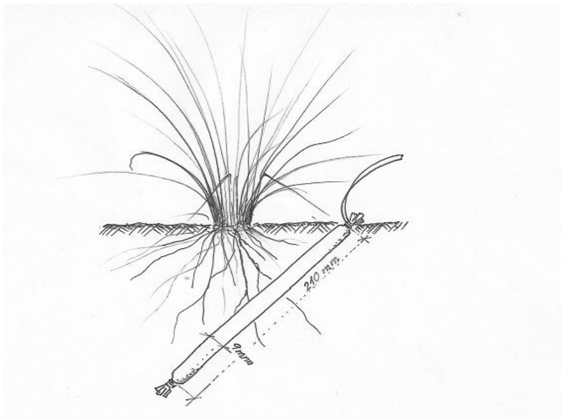
MATERIALS AND METHODS

The experimental plots were situated between 1090–1165m a.s.l in Almería which is province of Autonomous Community of Andalucía, Spain. These area extent of 82 000 m^2 and is located in Sierra de los Filabres Mountains. The mean annual rainfall of the region is of about 240 mm mostly concentrated in autumn and spring which is common in semiarid Mediterranean climate. The mean annual temperature is 13.9 °C. The studied soil, classified as Lithosol, has a loam to sandy clay texture (FAO-ISRIC and ISSS, 1998) and pH 6.5 and content of organic matter 1.6%. The vegetation cover of these areas is an open steppe dominated by *Stipa tenacissima* L. (coverage of up to 75%).

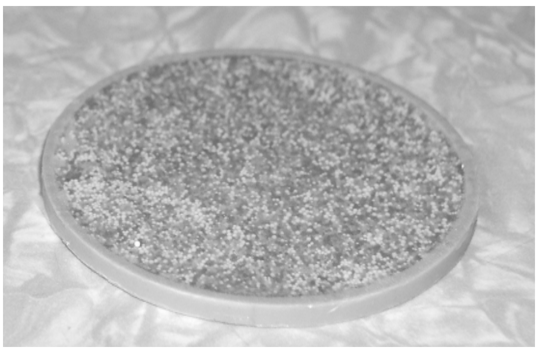
The availability (more or less in the case of ammonia-nitrogen) and movement of percolated nitrogen (mainly in the case of nitrate-nitrogen) was estimated in situ according to Binkley at Matson (1982) by the trapping of mineral N into the ion exchange resin (IER) inserted into two different types of special cover. Ion exchange resin stockings were prepared by placing ion exchange resin (CER, cation exchange resin No. Purolite C100E, and AER, anion exchange resin no. Purolite A520E) in cylindrical stockings (0.9 cm diameter by 21.3 cm long), made of fine nylon mesh (grid size of 42 µm) which were then stapled shut. The stockings contained either 11.2 g moist weight cation exchange resin (13.7 mL, 5.4 g dry weight) or 9.3 g moist weight anion exchange resin (13.7 ml, 4.9 g dry weight). Exchange sites of IER were saturated with Cl^- and Na^+ ions. The first set of CER- and AER-stockings were prepared for insertion into the soil as a "control" variant. Second set of stockings were coated with pure cellulose as the source of carbon and third set were coated with raw silk as the source of both, carbon and nitrogen to support microbial activities.

The first types of prepared IER stockings (bags) were randomly inserted into cylindrical holes (1.0 cm diameter) in the experimental plots. The holes were prepared by pushing a metal rode into a soil profile at a 45° angle to a soil depth of 15 cm (Fig. 1). This ion exchange resin bags can be placed into soil with minimal soil disturbance. This type of IER stocking were inserted to the soil on the research place in 12 repetitions. Another set of special annular flat cover (disc) for estimation of the movement of inorganic nitrogen was made from PVC tubes (diameter 7 cm, thickness 4 cm). Nylon mesh (grid size of 0.1 mm) was stuck on the PVC ring. Mixed IER (CER and AER in ratio 1:1) were then placed into the inner space of annular flat cover (Fig. 2). The annular flat IER cover was inserted into the soil in the depth of 30 cm in the experimental plot in 30 repetitions. Accumulation of N took 24-week over the 36 month period and it included wet season (October – April) and dry season (May – September).

For the quantification of NH_4^+-N and NO_3^--N trapped by the resin, the IER were allowed to dry at room temperature. Absorbed NH_4^+-N and NO_3^--N were evaluated from IER using 100 ml 1.7 M NaCl and determined by distillation and titration method (Peoples *et al.*, 1989). Results from the ion exchange resin bags were expressed as mg of NH_4^+-N.10 ml^{-1} IER and NO_3^--N.10 ml^{-1} IER. The results from the ion exchange resin discs were expressed as mg NH_4^+-N.m^{-2} and NO_3^--N.m^{-2}. Statistical evaluation



1: *Scheme of application of ion exchange resin (IER) in the first type of special cover, in fine nylon stockings*



2: *Second type of special cover - annular flat ion exchange resin cover which were inserted into the soil in the depth of 30* was performed by means of analysis of variance (ANOVA P < 0.05). When comparing the means, the Tukey's HSD procedure was used as a multiple range tests.

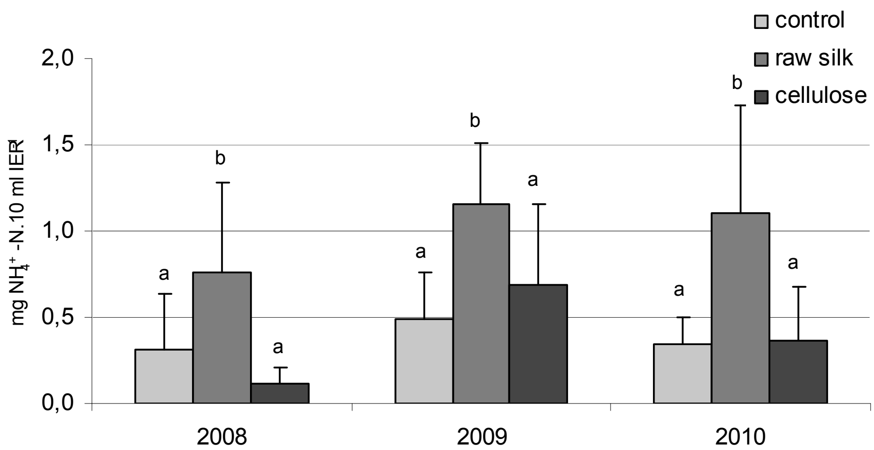
RESULTS AND DISCUSSION

Presented experiments are based on the estimation of nitrogen ion mobility and transport using ion capture into the structure of resin bags. The sensitivity of the resin bag method to ion mobility and transport may be useful for assessing N availability to plant roots, because the driving forces which affected the captured amount into the resin bags are nearly the same as in the case of nutrient uptake by roots (Binkley, 1984).

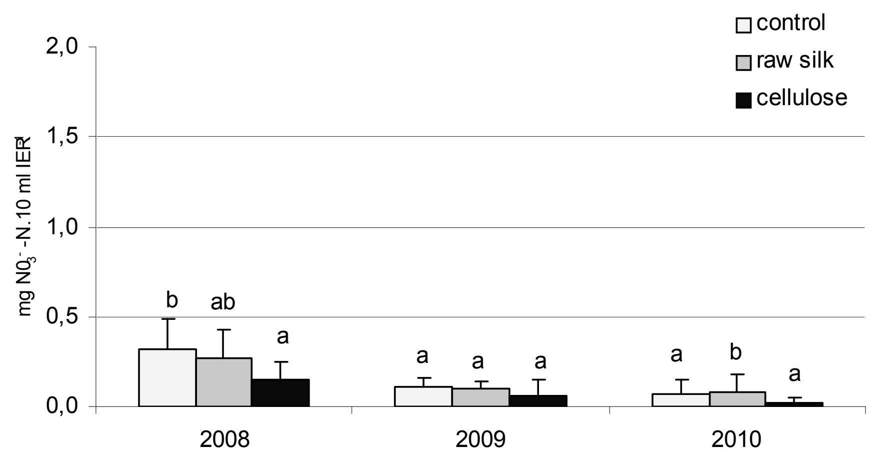
There exist several experiments based on using ion exchange resin in laboratory conditions but the laboratory incubations cannot reflect changes in temperature and moisture that occur in the field, which are crucial for the nutrient availability in arid and semiarid soils. The sum of captured ammonium and nitrate ions in ion exchange resins is directly proportional to the availability of given key nutrient in its own environment. In other words, this is amount proportional to the quantity which was released (or lost) from the enclosed internal cycles. The size of standard deviation illustrates the great variability of results which is typical for field studies of nitrogen transformations.

The ammonium ions are less mobile in the soil profile and they are derived mainly from microbial transformation of organic nitrogen. Therefore the ion exchange resins applied into cylindrical holes situated in the upper soil layer (to a depth of 15 cm) have a higher amount of trapped ammonium forms of nitrogen than nitrate forms (Fig. 3 and 4).

The availability of soil ammonia-nitrogen in control stockings, and after the addition of raw silk was not statistically different between studied years. Significant differences were detected only within

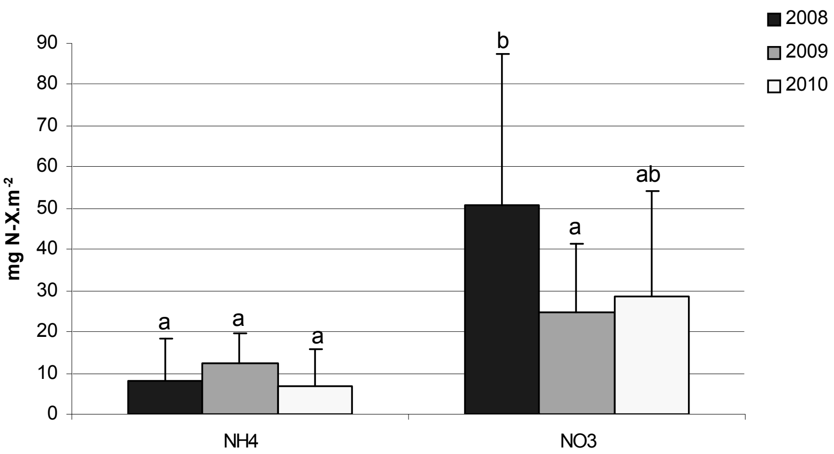


3: *The availability of soil ammonia-nitrogen from the upper soil horizons to a depth of 15 cm. Mean values from 12 replicates (bars) and SD (lines) are given. Different letters indicate significant differences (P < 0.05).*

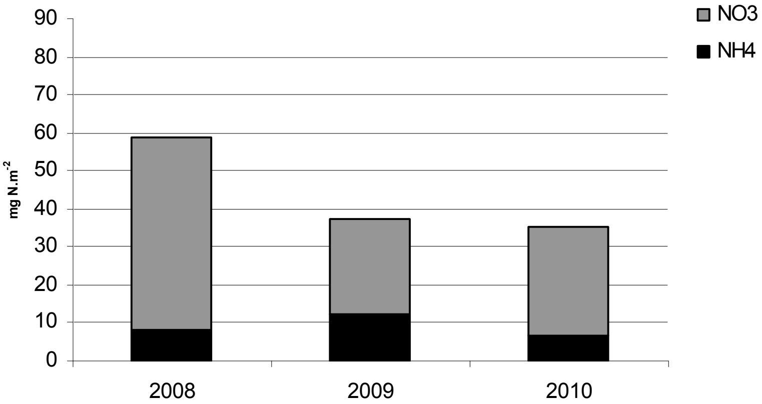


4: *The availability of soil nitrate-nitrogen from the upper soil horizons to a depth of 15 cm. Mean values from 12 replicates (bars) and SD (lines) are given. Different letters indicate significant differences (P < 0.05)* the variant with the addition of cellulose ($P < 0.05$). About the availability of soil nitrate-nitrogen, the statistical differences between types of IER stockings were found in 2008. Only the nitrate-nitrogen captured by IER with raw silk was significantly higher in 2010.

Higher amounts of captured ammonia form of nitrogen were estimated after the addition of raw silk. The amount of captured ammonia-nitrogen after addition of raw silk was significantly higher in all years. It can be concluded that the addition of raw silk supports the release of ammonia-nitrogen and restricts the rate of nitrate-nitrogen. However, the addition of cellulose can reduce the capture of nitrate-nitrogen. This biopolymer as a substitute of plant residues offer the surplus of carbon and energy source for microorganisms and thus cause higher demands of soil microorganisms about the sources of soil nitrogen. Binkley (1984) present that the addition of cellulose to the soils reduced N availability in unfertilized and fertilized soils. Tůma *et al.* (2010) confirm that the microbial competition for available nitrogen is very high after the addition of cellulose, which consequently restrict the rate of mineral nitrogen trapped into the ion exchange resin. This was also evident in our experiment. The only exception occurred in 2009 when the capture of ammonia-nitrogen was higher in variant with cellulose than in control. It is probably related to the lowest rainfall, high temperature, low humidity and high evapotranspiration in this year. Lajtha (1988) established that the wet season values of captured ammonia-nitrogen are significantly greater than dry season values. Only accumulation during the wet season showed significant differences among sites, thus stressing the role of field water regime in interpreting resin accumulation results. Jiangming *et al.* (1997) confirm the significantly seasonal variation in a pine forest of Dinghushan, with the highest in spring and the lowest in summer. Statistically significant differences were found out in content of nitrate-nitrogen captured by the different types of IER stockings. However, only in 2009, there were no significant differences in content of nitrate-nitrogen captured by types of IER stockings (Fig. 4).



5: *The movement of soil mineral nitrogen in the form of ammonia- and nitrate-nitrogen from the upper soil horizons to a depth of 30 cm. Mean values from 30 replicates (bars) and SD (lines) are given. Different letters indicate significant differences ($P < 0.05$).*



6: *The movement of soil mineral nitrogen in the form of ammonia- and nitrate-nitrogen from the upper soil horizons to a depth of 30 cm*

Most of the captured nitrate ions into the ion exchange resins come probably from leaching percolates. This confirms the higher detection of nitrate-nitrogen in ion exchange resins from the second type of special cover (discs) inserted into the soil in depth of 30 cm (Fig. 5 and 6).

High levels of total mineral nitrogen captured by ion exchange resin discs were found in 2008. The portion of nitrate-nitrogen was 84% of the total content of mineral nitrogen. Low amounts of ammonia-nitrogen indicated low availability of ammonia-nitrogen and probably high nitrogen use efficiency by the soil biota and consequently low nitrate movement into the deeper soil horizons. The capture of soil ammonia-nitrogen was not statistically different between all studied years. High levels of nitrate-nitrogen captured by ion exchange resin discs were found in 2008. There were statistically significant differences between all studied years. The low detection of nitrate nitrogen in 2008 is probably related to the lowest rainfall.

CONCLUSIONS

This experiment demonstrated the effect of addition of readily available carbon and nitrogen in the form of cellulose or raw silk on availability of mineral nitrogen directly in soil environment. It can be concluded that the addition of raw silk supports the release of ammonia-nitrogen and restricts the rate of nitrate-nitrogen. The addition of cellulose can reduce the capture of nitrate-nitrogen. The higher detection of nitrate-nitrogen in ion exchange resins inserted into the soil in depth of 30 cm confirms, that the nitrate ions are more mobile in the soil profile.

SUMMARY

This experiment demonstrated the effect of addition of carbon in the form of cellulose or carbon and nitrogen in the form of raw silk and zero addition (control variant) on availability of mineral nitrogen directly in soil environment. Measurements of N availability and movement of percolated nitrogen under field conditions include the trapping of mineral N into the ion exchange resin (IER) inserted into two different types of special cover (Binkley at Matson, 1982). Absorbed NH_4^+-N and NO_3^- -N were evaluated by distillation and titration method (Peoples *et al.*, 1989). The sensitivity of the resin bag method to ion mobility and transport may be useful for assessing N availability to plant roots, because the driving forces which affected the captured amount into the resin bags are nearly the same as in the case of nutrient uptake by roots. The sum of captured ammonium and nitrate ions in ion exchange resins is directly proportional to the availability of given key nutrient in its own environment. The ammonium ions are less mobile in the soil profile and they are derived mainly from microbial transformation of organic nitrogen. Therefore the ion exchange resins holes situated in the upper soil layer (to a depth of 15 cm) have a higher amount of trapped ammonium forms of nitrogen than nitrate forms. On the other hand, most of the captured nitrate ions into the ion exchange resins come from leaking percolates. This confirms the higher detection of nitrate-nitrogen in ion exchange resins from the second type of special cover (discs) inserted into the soil in depth of 30 cm. The addition of raw silk supports the release of ammonia-nitrogen and restricts the rate of nitrate-nitrogen. However, the microbial competition for available nitrogen is very high after the addition of cellulose, which consequently restricts the rate of mineral nitrogen trapped into the ion exchange resin. This biopolymer as a substitute of plant residues offer the surplus of carbon and energy source for microorganisms and thus cause higher demands of soil microorganisms about the sources of soil nitrogen.

Acknowledgements

This study was supported by the Research plan No. MSM6215648905 financed by the Ministry of Education, Youth and Sports of the Czech Republic and by the Research plan No. QH72039 financed by the Ministry of Agriculture of the Czech Republic.

REFERENCES

BINKLEY, D., 1984: Ion exchange resin bags: factor affecting estimates of nitrogen availability. *Soil Science Society of America Journal*, 48, p. 1181–1184, ISSN 0361-5995.

BINKLEY, D., MATSON, P., 1983: Ion exchange resin bag method for assessing forest soil nitrogen availability. *Soil Science Society of America Journal*, 47, p. 1050–1052, ISSN 0361-5995.

FROUZ, J., NOVÁKOVÁ, A., 2005: Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development. *Geoderma*, 129, p. 54–64, ISSN 0016-7061.

JIANMING, M., MENGDE, Y., GUOHUI, K., 1997: The dynamics of soil NH_4^+- N and NO_3^--N in a pine forest of Dinghushan, as assessed by ion exchange

resin bag method. *Acta Phytoecologica Sinica*, 21(4), p. 335–341, ISSN 1005-264X.
KAUR, B., GUPTA, S. R., SINGH, G., 2000: Soil carbon, microbial activity and nitrogen availability in agroforestry system on moderately alkaline soils in northern India. *Applied Soil Ecology*, 15, p. 283–294. ISSN 0929-1393.
LAJTHA, K., 1988: The use of ion-exchange resin bags for measuring nutrient availability in an arid ecosystem. *Plant and Soil*, 105, p. 105–111, ISSN 0032-079X.
MAESTRE, F., CORTINA, J., 2006: Ecosystem structure and soil-surface conditions drive the variability in the foliar δ^{13} C and δ^{15} N of *Stipa tenacissima* in semiarid Mediterranean steppes. *Ecologycal Research*, 21, p. 44–53, ISSN 0912-3814.
PEOPLES, M. B., FAIZAH, A. W., RERKASEN, B., HERRIDGE, D. F., 1989: *Methods for evaluating nitrogen fixation by nodulated legumes in the field*. Aciar Monograph, 11, p. 76, ISSN 1447-090X
RUTIGLIANO, F. A., CASTALDI, S., ASCOLI, R. D., PAPA, S., CARFORA, A., MARZAIOLI, R., FIORETTO, A., 2009: Soil activities related to nitrogen cycle under three plant cover types in Mediterranean environment. *Applied Soil Ekology*, 43, p. 40–46, ISSN 0929-1393.
TUMA, I., HOLUB, P., ZÁHORA, J., FIALA, K., 2010: Effect of enhanced nitrogen input on release of nutrients and nutrient availability in stand of tall fern *Athyrium distentifolium*. *Geophysical Research Abstract* [online], Vol. 12, April [cit. EGU 2010–8266]. Dostupný z www: <http: geophysical-research-abstracts.net/gra_volume_12.pdf>. ISSN 1607-7962.

Address

Ing. Irena Novosádová, Zemědělský výzkum, spol. s r. o. Troubsko, Zahradní 1, 664 41 Troubsko, Česká republika, Ing. Jaroslav Záhora, CSc., Ústav agrochemie, půdoznalství, mikrobiologie a výživy rostlin, Mendelova univerzita v Brně, Zemědělská 1, Brno 613 00, Česká republika, José Damian Ruiz-Sinoga, Department of Physical Geography, University of Malaga, Campus de Teatinos, Málaga 29071, Spain, e-mail: novosadova@vupt.cz, zahora@mendelu.cz, Sinoga@uma.es