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THE EFFICIENCY OF NUTRIENT UTILIZATION BY PERMANENT GRASSLAND IN THE KAMENIČKY LOCALITY

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Abstract

NAWRATH ADAM, ELBL JAKUB, KINTL ANTONÍN, ZÁHORA JAROSLAV, SKLÁDANKA JIŘÍ: *The efficiency of nutrient utilization by permanent grassland in the Kameničky locality.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 6, pp. 1799–1806

This work presents the analysis of the effect of grassland management on leaching of mineral nitrogen from rhizosphere and plant biomass production in area of our interest. The determined values show the influence of fertilization and species composition of permanent grassland on leaching of mineral nitrogen and biomass production. The values were determined during field and laboratory experiments. The highest availability of N_{min} in the field experiment was detected in the variant N90+PK; the lowest availability of N_{min} was recorded in the PK variant. The difference was statistically significant ($P < 0.05$). In the pot experiment, the highest loss of N_{min} was recorded in the variant with N180+PK (16.88 $mg.dm^{-3}$) with the difference being statistically significant as compared with the other variants ($P < 0.05$). Measured values of the availability of mineral nitrogen and plant biomass production indicate the importance of species composition at the establishment of new grassland. For example, species composition of permanent grassland has a great importance at the establishment of new grassland on erosion-affected soils in protection areas of drinking water. Because selected species of grasses can immobilize nitrogen in their bodies, thus preventing it's leaching from the soil.

mineral nitrogen, permanent grassland, primary production, fertilization

Meadows and pastures are constituents of harmonic cultural landscape from times immemorial. Grasslands represent an important landscape element, which can significantly facilitate stabilization of broader ecological linkages in the landscape if their biological and ecological regularities are identified and respected (Heger *et al.*, 2007). Permanent grasslands occupy over 23% of farmland in the Czech Republic and their area has been ever increasing. According to the Czech Office for Surveying, Mapping and Cadastre (COSMC), the area of permanent grasslands increased in 2010 by more than 3,000 ha. From the non-market perspective, grassland farming represents a great potential for ecological functions, which include species biodiversity, erosion control, infiltration and retention of water in the landscape and the function of biological filter (Hrevušová, 2011).

The most important pratotechnical measures are regular cutting and fertilizing, which is the main production factor together with the sufficient amount of water. Industrial agriculture has been increasing nitrogen inputs into biosphere by ever-higher doses of mineral fertilizers, which subsequently increases the amount of nitrates leached into drains and ground water (Úlehlová, 1989). Contrary to natural ecosystems, agricultural ecosystems are open systems with high nitrogen flows both on the input and on the output (Skořepová *et al.*, 1999). Nitrogen leaving the ecosystem is therefore a very good indicator of disturbed bonds within the N cycling. From the perspective of water manager, extremely important is the output from the ecosystem oversaturated with nitrogen in the form of nitrates (Záhora, 2011). If the nitrogen losses from the ecosystem are excessive, the efficiency of N-fertilization is limited by other factors. Then the fertilization would become ineffective and would conversely load the ecosystem with excessive doping of nutrients. Intensive fertilization may also lead to negative succession and hence to the reduced production of above-ground biomass (Hanzes *et al*., 2005). A no less serious consequent of excessive fertilization is also suppressed species diversity, which puts limitations on the capacity of grasslands to fulfil a range of non-productive functions.

The paper aims to assess the effect of fertilization on the availability and loss of nutrients and on the yields of grass stands. Its objective is to establish an optimum fertilizer dose, which would ensure sufficiently high yields at a simultaneous minimization of nutrient losses by leaching into ground water.

MATERIAL AND METHODS

Efficiency of nutrient utilization by permanent grassland was ascertained in the field and laboratory experiments at the Faculty of Agronomy, Mendel University in Brno. The field experiment was conducted in the research locality of Kameničky and the laboratory experiment was established at the workplace of Microbiology.

Field experiment

The experiment was established on research plots in the cadastre of the village Kameničky with the permanent stand of *Sanguisorba-Festucetum comutatae* association. The village of Kameničky is situated in the Protected Landscape Area of Žďárské vrchy Hills. The site has a SW aspect and is situated on a slope with the gradient of 3°. Mean annual temperature (1951–2000) is 5.8 °C and mean annual precipitation amount is 758.4mm. Soil type is acidic Luvic Stagnosol on the gneiss diluvium. The experiment was designed by using the method of split compartments in four repetitions. The evaluated factor was fertilization (no fertilization – control, PK fertilization, N90+PK fertilization and N180+PK fertilization. The stands were exploited in the system of three cuts. The contents of available nutrients established by the Mehlich III method are presented in Tab. I.

Nitrogen was supplied in the form of ammonium nitrate with limestone (LAV 27%) at a total dose of $90\,kg.ha^{-1}N$ resp. $180\,kg.ha^{-1}N$. In the mode of three cuts, the nitrogen dose was applied in three terms (1/3 in spring, 1/3 after 1st cut and 1/3 after 2nd cut). Potassic and phosphoric fertilizers were applied in spring. Phosphorus was applied in the form of Hyperkorn (26%) at 30 $kg.ha^{-1}$ P and potassium was applied in the form of potassium salt (60%) at 60 $kg.ha^{-1}$ K. The stands were harvested in three terms (early June, early August and early October). The grass was harvested by the mower Model MF-70 equipped with a cutting bar (engagement 1.2 m).

Characteristics assessed in our experiment included dry matter yields for the entire growing season and availability of nutrients in the rhizosphere. In order to establish the share of individual species in the harvested forage, samples were taken of above-ground biomass from permanently staked plots (0.5 m^2). The samples of the above-ground forage biomass were sorted out into individual species and dried at 60 °C. Subsequently, their dry weight was established and the proportions of individual species were expressed as percentages from the total weight of dry forage.

Availability and movement of N_{min} was tracked by means of the *in situ* application of mixed ionex (IER) beads composed of cation exchange resin (CER) and anion exchange resin (AER) beads in the ratio of 1:1 (preserving the 1:1 anex and catex ratio) in probes made of Uhelon fine nylon mesh (Silk & Progres, type 130 T, mesh size 42 µm). The probes were sewn together to form a roller of 18cm in length and one cm in diameter. Each probe was worked into the soil by means of a steel spike at an angle of 45° (Novosadová, 2011). Exposure time during the growing season was from 26 April 2012 to 11 November 2012. After 199 days of exposition, eight probes were removed from the soil profile and the absorbed ions were resorbed by applying 10% NaCl solution. Released ions were ascertained by distillation and titration method according to Peoples *et al*. (1989). The ammonium and nitrate forms were established separately with their sum in the respective variants representing the N_{min} capture (Elbl *et al*., 2012).

I: *Soil contents of available nutrients [$mg.kg^{-1}$] in 2012*

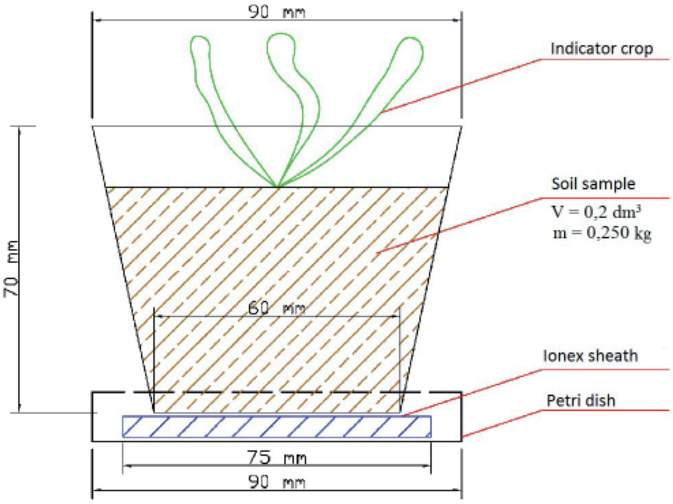
Fertilizer intensity	P	K	Ca	Mg	pH
No fertilizer	34,62	75,9	2064	177	4,6
PK	100,13	88,9	2597	197,7	4,7
N 90 + PK	89,84	89,8	2167	167,2	4,5
N 180 + PK	92,64	87,2	2406	183,7	4,6

Laboratory experiment

The laboratory experiment was implemented with using the soils sampled from the site in Kameničky. Main indicators of the capacity of the respective variants to utilize efficiently the applied nutrients were chosen to be the N_{min} loss and the biomass production.

Laboratory experiment scheme

The pot experiment was established to measure the N_{min} loss from the soil with the permanent grass stand. A model of the used pot is presented in Fig. 1. The individual pots were filled with soil (m = 250 g) collected from the Kameničky site. Indicator crop was *Lactuca sativa* L. Disturbed soil samples were taken on 26 April 2012 in accordance with CSN ISO 10 381-6. Prior to their application into the experimental containers, the collected samples were homogenized, sifted through a sieve with a mesh size of 2 mm, and pre-incubated for 30 days at a laboratory temperature (t ~ 20 °C).



1: *Design of the laboratory experiment*

The experiment consisted of four fertilization variants, each with three repetitions (V1 – no fertilizer, V2 – P and K fertilization, V3 – N90 and PK fertilization and V4 – N180 and PK fertilization).

Course of experiment exposition in the growth box

The loss of N_{min} was monitored in each experimental container under controlled conditions (t = 22 °C, light intensity 300μmol/$m^{-1}s^{-1}$, light period 16/8) in the growth box for 36 days. Indicator crop seeds (8 pcs) were sown into each previously prepared container and all containers were irrigated with 15 ml of distilled water per day for a period of ten days. After that time, the sown seeds germinated and only one plant was left in each container. In the remaining time of the experiment, the plants were irrigated with 30 ml of distilled water for three weekdays (Mo, Tue, Fri).

Determination of N_{min} loss

The N_{min} capture was measured with using the mixed ionex beads (IER, Ion Exchange Resin) composed of cation (CER, Cation Exchange Resin) and anion (AER, Anion Exchange Resin) beads. Individual IER sheaths consisted of Novodur tubing rings, each of 75 mm in diameter and 5mm wall thickness. The UHELON mesh (fine nylon) was attached on both sides of the discs so that the discs could be filled with the above-mentioned CER and AER mixture (1:1). The resorption of captured N_{min} and its subsequent determination were made similarly as in the field experiment. The conversion of the N_{min} of ionex to the mg in dm^3 of soil was conducted according to the algorithm of Novosadová *et al.* (2011).

Determination of above-ground and under-ground plant biomass production

The above-ground and under-ground biomass of the indicator crop was sampled separately from all repetitions of individual experimental variants. Dry weight was established according to Elbl *et al.* (2013), which for the paper purposes represented the value of above-ground and under-ground biomass production.

Statistical evaluation

The obtained measurements were statistically evaluated with using the single-factor analysis of variance (ANOVA). In addition, we calculated the least significant differences (LSD) at a significance level of 5% ($p < 0.05$) with Post-Hoc Tuckey's HSD test. The statistical analysis was performed with using the STATISTICA 10 programme and the data were graphically processed in Excel 2010 (MS OFFICE).

RESULTS AND DISCUSSION

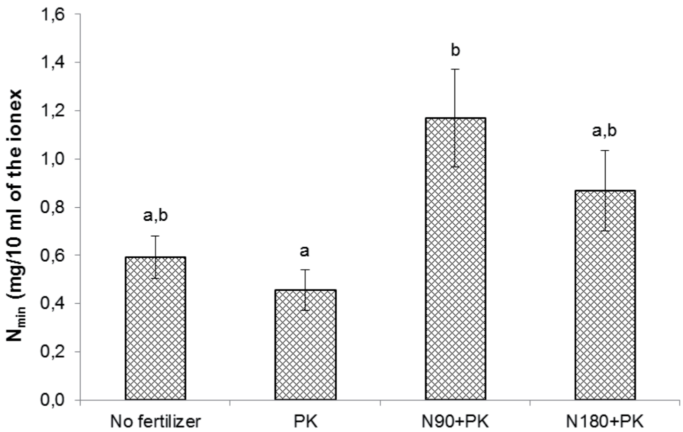
Field experiment

Availability of mineral nitrogen in the rhizosphere of grassland belonging in the association of Sanguisorba-Festucetum comutatae

Availability of N_{min} in the rhizosphere, i.e. at a depth of up to 18cm was monitored in the period from 26 April 2012 – 11 November 2012 (spring-autumn). N_{min} capture values found on ionex beads are graphically illustrated in Fig. 2.

The highest and lowest N_{min} availability was recorded in the N90+PK variant and in the PK variant, respectively. The difference between these variants was statistically significant ($P < 0.05$). Compared with the N90+PK variant, the N180+PK variant showed impaired availability of N_{min} in spite of the fact that it was doped with a double amount of nitrogen. Since the N180+PK variant did not exhibit any distinctive increase of yields, the answer should be sought in the species composition (Tab. II).

Grasses were the most represented agro-botanical group in the N180+PK variant; dominant



2: *The amount of IER-trapped soil N_{min}; different letters indicate significant differences between the respective variants*

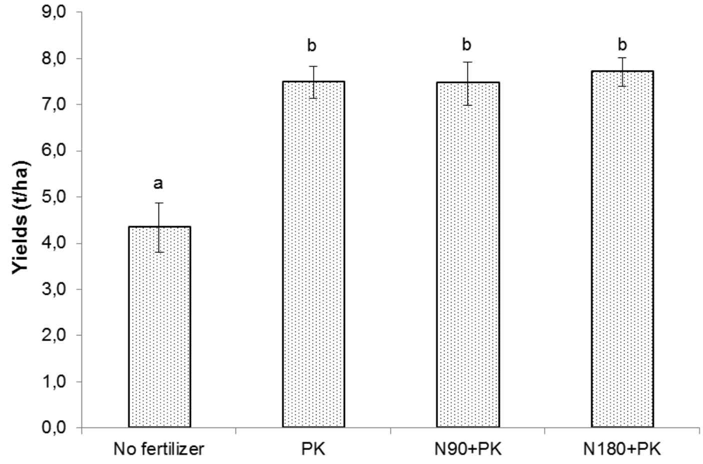
II: *Representation (%) of dominant species and agro-botanical groups in the stand in 2012 in the respective variants*

Representation (%) of dominant species and agro-botanical groups in the stand				
Species, groups/Variant	**No fertilizer**	**PK**	**N90+PK**	**N180+PK**
Festuca rubra	6,4	2,3	4,4	1,5
Poa pratensis	2,0	4,8	3,9	10,3
Alopecurus pratensis	0,7	12,4	21,0	24,8
Sanquisorba officinalis	1,6	13,8	8,3	4,4
Carex ssp.	32,2	2,5	0,2	0,2
Ranunculus acris	3,2	15,5	1,9	0,7
Ranunculus auricomus	15,1	8,2	8,7	13,7
Bistorta major	9,9	8,1	23,4	16,5
Juncus conglomeratus	4,3	4,2	7,5	8,5
Rumex acetosella	0,3	0,5	1,7	3,2
Trifolium repens	2,9	16,8	5,3	1,8
Grasses	40,8	27,0	49,7	53,4
Clovers	6,9	22,6	9,1	2,4
Other herbs	52,3	50,5	41,1	44,1

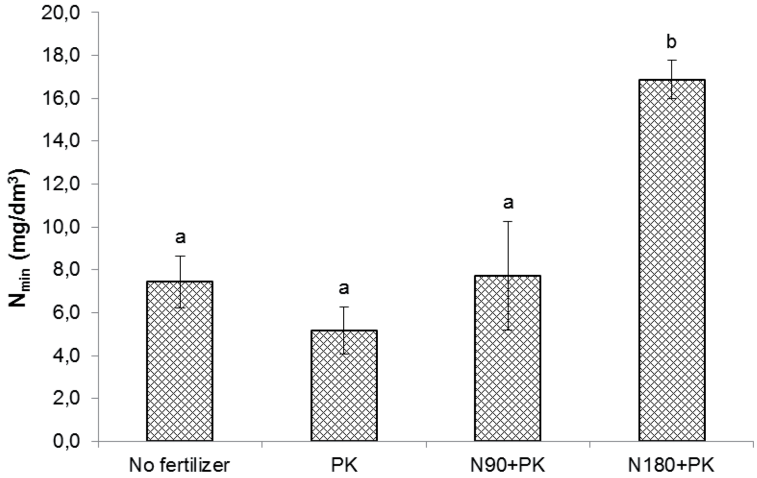
species were *Alopecurus pratensis* and *Poa pratensis*. High-production grasses can fix in their biomass a considerable amount of nitrogen, which might explain the decreased availability of N_{min} in this variant. It is a well-known fact that the species of *Alopecurus pratensis* is capable of utilizing effectively nitrogen doses up to 300 $kg.ha^{-1}$. The capacity of *Poa pratensis* to reduce the amount of leached NO_3^- is known as well (Bouman *et al.*, 2010). High nitrogen doses often result in the occurrence of a so-called undesirable flora of nitrophilous species. The N-doped variants were demonstrated to exhibit a higher incidence of *Rumex acetosa* and *Polygonum bistorta* than the variants without the nitrogen fertilization. The lowest availability of mineral nitrogen was recorded in the PK variant. An explanation for this fact appears to be the high yield of primary production and the representation of individual agro-botanical groups (Fig. 3, Tab. II).

Above-ground biomass production on the research locality

Comparing the primary production of above-ground biomass (Fig. 3) and the availability of N_{min} in the rhizosphere (Fig. 2), we can see that the PK variant of fertilization exhibited the lowest N_{min} availability (0.46 mg/10 ml of ionex) while the production of above-ground biomass was comparable with the N90+PK and N180+PK variants. This indicates that the variant showed the same production capacity with a simultaneous maximum utilization of N_{min} available in the rhizosphere. The high share of clover crops in the stand (22.6%) presumably contributed to the high yields. The capacity of clover crops to fix atmospheric nitrogen into the form utilizable not only for their own need but also for other plants in the community is well known. Although there are studies (Bouman *et al.*, 2010) claiming that a high share of legumes can increase the amount of available nitrogen, it was not the



3: *Primary production of biomass in the study area in 2012*



4: *Loss of N_{min} from experimental containers in the respective variants*

case of our experiment. We found out that nitrogen produced by clover crops was readily utilized by other plants in the association due to the absence of nitrogen fertilization, which resulted in high yields. This correlation between the share of clover crops and yields was demonstrated also by Skládanka *et al.* (2008).

As to the production of plant biomass, a statistically significant difference ($P < 0.05$) was found between the unfertilized variant (control) with the lowest yields and the variants doped with fertilizers (PK, N90+PK and N180+PK). The individual fertilized variants showed yields comparable with the negligible increase in the N180+PK variant

Laboratory experiment

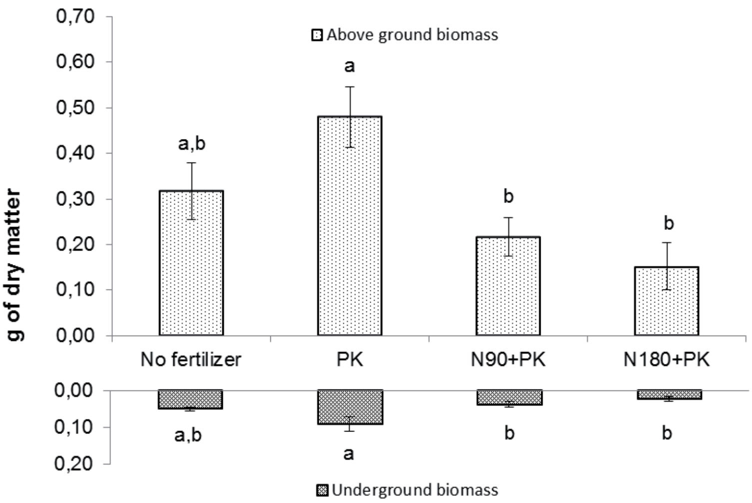
We measured the loss of N_{min} during the pot experiment and ascertained the production of above-ground and under-ground biomass upon its end.

Loss of mineral nitrogen from experimental containers

The highest loss of mineral nitrogen recorded during the pot experiment was found in the N180+PK variant (16.88 $mg.dm^{-3}$) with the difference being statistically significant ($P < 0.05$) as compared with the other variants. It was obvious in the variants with the highest N_{min} loss that the soil was saturated with mineral nitrogen, which could not be utilized by the indicator crop. The lowest N_{min} loss was recorded in the PK variant (5.17 $mg.dm^{-3}$).

The reason was the presence of phosphorus as limiting element for the utilization of N_{min} by soil organisms in the rhizosphere. Its increased availability results in the immobilization of free N_{min} in the bodies of microorganisms, and at the same time in its decomposition by metabolic processes of microorganisms into a more readily utilizable ammonium form (Šimek, 2007; Novosadová *et al.*, 2011; Záhora *et al.*, 2011).

The N_{min} loss is expressed as a sum of the weighted means of ammonium nitrogen and nitrate nitrogen



5: *Production of above-ground and under-ground biomass in g of DM of Lactuca sativa*

trapped in the respective variants. The values of captured N in mg were converted to dm^3 of soil.

Production of above-ground and under-ground biomass

The amount of leached nitrogen was closely connected also with the increased biomass of *Lactuca sativa*. The greatest increase of both above-ground (0.48 g) and under-ground (0.09 g – see Fig. 5) biomass was recorded in the PK variant. The lowest increase was found in the N180+PK variant (0.15 g and 0.02 g, resp.). Differences between the variants were significant ($P < 0.05$). A significant difference was also observed between the PK and N90+PK variants. Compared with the N180+PK, a high amount of biomass was recorded in the unfertilized variant. The results suggest that *Lactuca sativa* is a species distinctly sensitive to the soil supply of available nutrients, the growth of which is suppressed by the excessive doses of nutrients. D'Antuono *et al.* (2001) demonstrated the same fact and observed positive influence of fertilization on *Lactuca sativa* only to a maximum N dose 80 $kg.ha^{-1}$. The finding is further corroborated by comparing biomass increase in the pot experiment with meadow stand yields where the higher supply of nitrogen supported the growth in the meadow stand while the biomass increase in *Lactuca sativa* was decreasing. The reason was primarily the high representation of grasses capable of utilizing the high nitrogen doses for their growth, contrary to *Lactuca sativa*. In both cases, however, the growth was decidedly boosted by PK fertilization, probably due to the low content of available phosphorus in local acidic soils in which a so-called degeneration of phosphorus occurs, which is then fixed into forms not available to plants.

CONCLUSION

Based on the conducted field and laboratory inquiries, the authors can state that the loss of mineral nitrogen from the soil environment of permanent grassland is most affected by fertilization and species composition of the stand in question. The facts appear quite clear but they become very important in situations when the strategies of soil protection against the loss of nutrients and erosion have to be revaluated. The reason is that arable land is increasingly being converted into permanent grasslands. The effort is particularly obvious in the protection zones of water sources or in areas threatened by erosion. This is why the results of our study represent a possible theoretical basis e.g. for the planning of permanent grasslands in exposed areas. The most appropriate measure appears to be the PK fertilization when high yields are achieved even with the absence of N fertilizer and at the same time, the nutrients are not leached into sub-surface waters. In localities extremely endangered by the leakage of nitrates or in the protection zones of water sources, grass mixtures should include species such as *Alopecurus pratensis*, *Phleum pratense* or *Lolium perenne*, capable of immobilizing large amounts of nitrogen and thus preventing its loss.

SUMMARY

The objective of this study was to assess the effect of fertilization on the availability of nutrients and grassland yields, and thus to establish an optimal dose of fertilizers to secure sufficiently high yields and to minimize at the same time the losses of nutrients due to their leaching into ground water. Efficiency of nutrient utilization by permanent grassland was ascertained in the field and laboratory experiments at the Faculty of Agronomy, Mendel University in Brno. The field trial was conducted

in the Kameničky research locality. The assessed factor was fertilization in the following variants: no fertilization (control), PK fertilization, N90+PK fertilization and N180+PK fertilization. The stands were exploited in the system of three cuts. Evaluated characteristics included dry matter yields for the entire growing season and availability of nutrients in the rhizosphere determined by means of using a fine nylon mesh probe with mixed ionex beads. The laboratory experiment was established at the workplace of Microbiology and was conducted with using soils sampled from the Kameničky locality. Indicators of the capability of individual variants to utilize nutrients effectively were chosen to be the loss of N_{min} and the production of *Lactuca sativa* biomass.
The highest availability of N_{min} was detected in the variant N90+PK; the lowest availability of N_{min} was recorded in the PK variant. The difference was statistically significant ($P < 0.05$). The PK variant of fertilization showed forage production comparable with the nitrogen-doped variants the reason being a high representation of clover crops in the stand (22.6%), capable of binding nitrogen not only for their own need but also for the needs of other plants in the community.
In the pot experiment, the highest loss of N_{min} was recorded in the variant with N180+PK (16.88 $mg.dm^{-3}$) with the difference being statistically significant as compared with the other variants ($P < 0.05$). The highest increase of *Lactuca sativa* biomass was observed in the PK variant, which exhibited also the lowest N_{min} loss. Nitrogen in this variant was fully used for the growth with a minimum risk of losses due to leaching.

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