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Effect of organic amendments on nitrate leaching mitigation in a sandy loam soil of Shkodra district, Albania

Erdona Demiraj,[1] Angela Libutti,[2] Jamarbër Malltezi,[1] Evan Rroço,[3] Ferdi Brahushi,[1] Massimo Monteleone,[2] Sulejman Sulçe[1]

[1]Department of Agro-Environment and Ecology, Faculty of Agriculture and Environment, Agriculture University of Tirana, Albania; [2]Department of Science of Agriculture, Food and Environment, University of Foggia, Italy; [3]Department of Plant Science and Technologies, Faculty of Agriculture and Environment, Agriculture University of Tirana, Albania

Abstract

European lacustrine systems are frequently exposed to nitrate (NO_3^-) pollution causing eutrophication processes. An example of these lakes is Shkodra Lake, a large, shallow lake shared by Albania and Montenegro, in the Balkans Peninsula. Shkodra Lake is a natural sink that collects NO_3^- from agricultural activities, widely diffused in the surrounding area. The additions of wheat straw and biochar have been suggested to increase soil NO_3^- retention of agricultural lands. To better understand the role of these two organic soil amendments in mitigating NO_3^- leaching from arable lands, a pot experiment using a representative sandy loam soil of the Skodra Lake basin was performed. More specifically, a greenhouse experiment with *Lolium multiflorum* L. and *Zea mays* L., was carried out for three months, to evaluate the concentrations of NO_3^--N in leachate and the cumulative leaching losses of NO_3^--N, after wheat straw (10 Mg ha^{-1}) and biochar (10 Mg ha^{-1}) soil addition, under the same rate of NPK fertiliser (300 kg ha^{-1}). The effect of the two organic amendments on nitrate retention, was evaluated according to two methods: i) Soil NO_3^--N leaching with distilled water; and ii) Soil NO_3^--N extraction with 2*M* KCl. The leached NO_3^--N and the «*Potentially Leachable*» NO_3^--N (2*M* KCl extraction) were respectively determined. N uptake by plants, as well as the Nitrogen Use Efficiency were also calculated. A retention effect on nitrate was found in *Lolium multiflorum* L. and wheat straw treatments compared to control, by reducing leached NO_3^--N almost to 35%. In SBFL (soil+biochar+fertiliser+*Lolium*) treatment, biochar effectively reduced the total amount of nitrate in leachate of 27% and 26% compared to SFL (soil+fertiliser+*Lolium*) and SSFL (soil+straw+fertiliser+*Lolium*) treatments, respectively. The *potentially leachable* NO_3^--N was two to four times higher than the leached NO_3^--N. The amount of *potentially leachable* NO_3^--N per hectare ranged from 220 in SL (soil+*Lolium*) treatment, to 500 kg ha^{-1} in SFL. N plant uptake values ranged from 18.16 mg kg^{-1} in the non- fertilised treatment to 58.06 mg kg^{-1} soil in SSFM (soil+straw+fertiliser+maize) treatment. The NUE showed a similar trend (from 0 in the non-fertilised treatment to 47.9 % in SSFM). Results indicated a mitigating action of biochar on leaching of NO_3^--N (leached up to 100 kg ha^{-1}), despite the retention effect of the two different amendments applied.

Correspondence: Angela Libutti, Department of Science of Agriculture, Food and Environment, University of Foggia, via Napoli 25,71122, Foggia, Italy.
Tel.: +39.0881.589128.
E-mail: angela.libutti@unifg.it

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Introduction

Nitrate (NO_3^-) pollution of surface and groundwater is considered one of the most important water quality issues worldwide (Nolan, 2001; Puckett *et al*., 2011). Since the end of the 1950s, NO_3^-concentrations in surface water and in groundwater is constantly increasing (Billen and Garnier, 1999; Birgand *et al*., 2007). Increase of NO_3^- concentration in surface water can produce an increase in primary production and therefore anoxic conditions, promoting the eutrophication of water bodies (Vitousek *et al*., 1997; Rivett *et al*., 2008). Lakes are among the most sensitive surface water bodies, where the load of macronutrients causes more and more frequent pollution and eutrophication processes (Durand *et al*., 2011). Eutrophication in European lakes has been a major concern for decades (Schindler, 2006). Nitrogen (N) concentrations in lakes vary widely, depending on the intensity of human perturbation of the land in the surrounding basin; N loading is also highly variable, depending on the relative contribution of terrestrial sources, such as fertilisation and its management. According to Durand *et al*. (2011), the annual N loads into the basin of 40 European lakes studied vary from <0.02 to 29 kg N ha^{-1}. The nutrient load delivered to aquatic ecosystems depends strongly on the hydrological processes. Especially, the relative importance of different water pathways in the transfer of the various N forms from terrestrial to aquatic systems plays an important role (Molenat and Gascuel, 2002; Jordan and Smith, 2005).

NO_3^- is highly soluble within the soil water solution, poorly adsorbed by the soil particles and therefore prone to be leached away by the water percolating along the soil profile. NO_3^- leaching depletes soil fertility, limits the nitrogen utilisation efficiency by the plants, reduces crop yields and represents a significant economic cost for farmers (Libutti and Monteleone, 2017). A fraction of the total N applied to agricultural land, that is not taken up by plant roots, could moves in soluble form from the soil to surface waters or migrates into groundwaters, polluting the water with nitrates, nitrites or ammonia, as well as reducing the economic efficiency of fertilisers (Vitousek *et al*., 1997; Carpenter *et al*., 1998). In 1991, the European Union (EU) adopted the Nitrates Directive 91/676/CEE, with the aim to protect water quality by preventing nitrate leaching from agricultural activities and promoting the adoption of a code for *Good agricultural practices* (Libutti and Monteleone, 2017). Although the Nitrates Directive has been implemented in all EU Member States and continues to deliver pollution control, diffuse nitrogen pollution remains problematic. The data on the quality of European water resources (EEA, 2015) highlight that water quality has improved, although concentrations of nutrients in many places are still high and affect the water status. In regions with intensive agriculture production, diffuse nitrogen pollution is still high, resulting in continued eutrophication problems. High NO_3^- concentration in waters also impacts on human health, because of the associated risk of diseases, like gastrointestinal cancer in adults (Wolfe and Patz, 2002) and methahemoglobinemia in infants (Ward *et al*., 2005). To prevent this potential human health hazard, the World Health Organisation (WHO) established a contaminant level that should not exceed 50 mg NO_3^- L^{-1} in drinking water (WHO, 2004).

In order to mitigate NO_3^- leaching from agricultural fields effective agronomic solution must be developed. Soil incorporation of wheat straw, is very long studied as an efficient tool to retain NO_3^- in the soil (Nishio *et al*., 2003; Huang *et al*., 2004; Shindo *et al*., 2005). The use of a relatively new carbonaceous product such as biochar, as a potential mean to increase this retention, could be also very useful to reduce NO_3^- losses from agricultural soils and improve nitrogen utilisation efficiency for a sustainable crop production (Laird *et al*., 2010; Singh *et al*., 2010; Libutti *et al*., 2016). Wheat (*Triticum aestivum L.*) straw residues are an agricultural by-product that plays an important role in building up soil organic matter, increase soil fertility (Lou *et al*., 2011; Tong *et al*., 2014; Monteleone *et al*., 2015) and enhance soil retention of inorganic nitrogen forms, especially nitrate (Thomsen and Christensen, 1998; Döring *et al*., 2005). These agronomic advantages can be accomplished by adding to the soil wheat straw residues, alone or mixed with mineral N fertiliser which are very important in improving the enzymatic activity of soil microorganisms (Garg and Bahl, 2008). Previous studies (Meisinger and Delgado, 2002) indicated that wheat straw residues induced a net N immobilisation during the initial stages, and released N at later stages, largely depending on several biotic and abiotic factors. It has been reported that application of crop residues reduces N losses due to a slower cycling pool that caused greater N retention in soil amended with crop residue (73%) than with N fertiliser (26%), (Delgado, 2002).

Biochar, a stable and recalcitrant organic carbon compound, is the charred by-product of a thermochemical process (pyrolysis or gasification), which consists essentially in the heating of agricultural biomass in the complete or near absence of oxygen, in order to capture combustible gases. The oils and gases from pyrolysis can be used for energy production; the biochar can provide a useful co-product for the improvement of many soil characteristics (Laird *et al*., 2009; Monlau *et al*., 2016).

A number of studies, carried out in open field (Steinbess *et al*., 2009; Haider *et al*., 2017) in pots experiment (Lehmann *et al*., 2003; Buecker *et al*., 2016), using leaching columns (Yao *et al*., 2012; Bradley *et al*., 2015; Kanthle *et al*., 2016) or laboratory experiments (Libutti *et al*., 2016), indicated that biochar is an effective option of nitrate leaching mitigation. However, the impact of crop residue and biochar addition on soil N retention in agricultural soils are poorly understood. In a field experiment including the addition to the soil of wheat straw, biochar pyrolysed from wheat straw, and wheat straw plus its biochar, Hu *et al*. (2014) found higher soil NO_3^- concentrations in the treatment with biochar. Oppositely, Chen *et al*. (2012), in a laboratory incubation experiment aiming to investigate the effects of direct incorporation of either wheat straw or its biochar into a cultivated soil, showed that biochar could not effectively immobilise NO_3^- in the soil. These contradictory results suggest that straw-based biochar might have different effects on N leaching and retention in heavily fertilised cropland soils and its property of nitrate leaching mitigation need to be studied and verified.

The aim of the present study was to evaluate the effect of two different soil organic amendments on NO_3^--N retention, according to two methods: i) soil NO_3^--N leaching with distilled water; and ii) soil NO_3^--N extraction with 2*M* KCl. More specifically, the effect of the addition to a representative sandy loam soil of the Skodra Lake basin (Albania) of wheat straw and biochar from wheat straw on the retention of soluble mineral form of N was tested in a pot experiment, under controlled conditions.

Materials and methods

The study area

Shkodra Lake, the largest lake in the Balkans Peninsula, is located about 20 km from the Adriatic cost (42° 04′ 03″ N; 19° 30′ 47″ E), on the border between Albania in the south and Montenegro in the north. It covers a surface from approximately 370 to 600 km^2 and has a volume from approximately 1.7 to 4 km^3, depending on the seasonal fluctuations between dry and wet periods. It is approximately 5 m above sea level and mean depth is about 5 m, but can be as much as 60 m in isolated sublacustrine groundwater springs (Skarbøvik *et al*., 2014). Because of the wide range of endemic, rare or endangered plant and animal species it gives shelter, Lake Shkodra and its extensive associated wetlands are listed as one of 24 transboundary wetland sites of International value (EEA 1995), and are internationally recognised as a site of significance and importance according to the Ramsar Convention (1995). The Montenegrin side of the basin of Shkodra Lake has been proclaimed a National Park in 1983, while the Albanian side is a protected area *Managed natural reserve*, since 2005. Due to its importance, the lake has been a subject of numerous investigations, providing numerous physical, chemical and biological background data including environmental studies (Perovic *et al*., 2004; Rakocevic-Nedovic and Hollert, 2005). It is an ecologically sensitive water body where the biodiversity is under anthropogenic pressing and water quality is threatened mainly from loading of macronutrients used in agriculture (Malollari *et al*., 2012). The watershed of Shkodra Lake is characterised by concave topography, with slopes that range between 3 and 10% and a surrounding average elevation of 770 m a.s.l. (Dhora, 2016). The climate in the study area is Mediterranean, with the highest and lowest temperatures occuring respectively in July-August and December-January and an annual average precipitation of 750 mm (over a 50-year period), concentrated in two rainy seasons, spring and autumn (IGEWE, 2016). The watershed is affected by a high erosion processes. As a result, the parent material of the surrounding agricultural fields is mainly colluvial, calcareous, porous and waterproof. Shkodra Lake is the receiving water body for several large rivers and numerous small streams draining the surrounding catchment area. Due to the karstic nature of the watershed geology, the lake also receives significant input from numerous sub-lacustrine springs, which deliver waters percolating through adjacent agricultural lands (Rastall *et al*., 2004).

Regarding the land use, on the Albanian side of Shkodra Lake, the main activities of the population are agriculture and animal husbandry; the lands are prevalently cultivated with arable, vegetables and fruits crops. The agricultural activity is very intensive and includes the use of high crops fertilisation. Fertilisers applied by farmers to crops are easily leached out from the soil by rainwater and irrigation water causing a deterioration of lake water quality. Beside the intensive agricultural activity in the fields north of Shkodra (Figure 1), the large amount of waste, urban water, brought to the lake negatively affects the water quality and intensifies eutrophication of the Lake too (Dhora *et al*., 2012; Dhora, 2016). Indeed, other serious problems of the area are linked to the solid waste dumped throughout the territory along the riverbanks and the outlet of the sewage waters from towns and villages directly to the lake, cause an enrichment of waters with nutrients and chemical detergents increasing the eutrophication of waters (Cullaj *et al*., 2005). Serving as an agricultural field, this part of the Lake is the most ecologically sensible and economically important one (Mesi, 2013).

Experimental design

The pot experiment was carried out at Greenhouse Research Station, Agriculture University of Tirana), Albania and the applied experimental treatments were following Table 1. Seven treatments (T) with four replications (r) were compared: T1 = bare soil (S); T2 = soil + *Lolium multiflorum* L. (SL); T3 = soil + wheat straw (SS); T4 = soil (S) + NPK fertilisation (F) + *Lolium multiflorum* L. (L); T5 = soil + wheat straw (SS) + NPK fertilisation (F) + *Lolium multiflorum* L. (L); T6 = soil + wheat Straw (SS) + NPK fertilisation (F) + *Zea mays* L. *(90 day cycle)* (M); T7 = soil (S) + biochar from wheat straw (B) + NPK fertilisation (F) + *Lolium multiflorum* L. (L). The soil used in the experiment was taken in Gruemira village (42° 09′ 52, 10″ N, 19° 30′ 59, 43″ E), part of the Koplik municipality (Figure 1) in Malesia e Madhe District, in a deluvional Regosol (Calcaric) (WRB, Updated, 2015). Koplik is a municipality in the North-Western Albania, close to the border of Montenegro. Its territory lay within the Lake Shkodra basin. The municipality has an overall area of 930 ha, out of which 690 ha of urban land, and manages 2.7 km of Shkodra Lakeshore. The soil sampled in Gruemira village is one of the representative soils of the fields north of Shkodra, together with Regosols (especially Calcaric Regosol) and Luvisols (mainly Calcaric). The soil was collected from the cultivated layer (0-30 cm depth) of a farmland, in five points along two crossing diagonals. Before experiment started, stones and crop residues were removed from the collected soil, which was well mixed, air dried, crushed, passed through a 5 mm sieve and mixed thoroughly. The soil was then used for filling the plastic pots (22 cm in diameter and 24 cm high) used in the

Figure 1. Map of Shkodra Lake and soil sampling place in Gruemira Village, as indicated by the red point AL_ED_001_Soil, with geographic coordinates.

Table 1. Experimental treatments.

Treatment	Bare soil T1/S	Soil + *Lolium multiflorum* L. T2/SL	Soil + wheat straw T3/SS	Soil + NPK + *Lolium multiflorum* L. T4/SFL	Soil + wheat straw + NPK + *Lolium multiflorum* L. T5/SSFL	Soil + wheat straw + NPK + Zea mays L. T6/SSFM	Soil + biochar + NPK + Lolium multiflorum L. T7/SBFL
Replication	S 1 r	SL1 r	SS 1 r	SFL 1 r	SSFL 1 r	SSFM 1 r	SBFL 1 r
	S 2 r	SL 2 r	SS 2 r	SFL 2 r	SSFL 2 r	SSFM 2 r	SBFL 2 r
	S 3 r	SL 3 r	SS 3 r	SFL 3 r	SSFL 3 r	SSFM 3 r	SBFL 3 r
	S 4 r	SL 4 r	SS 4 r	SFL4 r	SSFL 4 r	SSFM 4 r	SBFL 4 r

1 r, 2 r, 3 r, 4 r, replications for each experimental treatment.

experiment (Figure 2). The pots, bottom packed with washed gravel with a diameter of 1cm (1 kg pot^{-1}) and filled with soil (7 kg pot^{-1}), were arranged in the seven experimental treatments with four replicates.

In May 2015, T2, T4, T5 and T7 pots were planted with ryegrass (three grams of seeds pot^{-1}), T6 pots with maize (three seeds pot^{-1}). The choice of maize and ryegrass crops followed the options usually applied by farmers in the study area. These two crops were cultivated since the 90's by farmer cooperatives, due to the climate and the soil physico-chemical properties which created the most favorable conditions for an optimal crop yield performance.

NPK fertiliser (15% N:15% P_2O_5:15% K_2O) was applied at rate of 300 kg ha^{-1}. In T2, T4, T5 and T7 fertilisation of reygrass was divided into three doses, corresponding to 3.90 g pot^{-1} dose^{-1}. The first fertilisation was carried out at 2-3-leaf stage, 2 weeks after sowing. The second fertilisation, with the same amounts of fertiliser, was applied 1 week after first harvest, which was 1 month after planting, and the same procedure was followed for the third dose of fertiliser (1 week after second harvest, which was 2 months after planting). In T6, fertilisation of maize was divided into two doses, corresponding to 5.80 g pot^{-1} dose^{-1}. The first fertilisation was directly applied after planting and the second at the 8-leaf stage. Fertilisation was carried out according to the local standard farming techniques as reported by INSTAT and Regional Directorate of Agriculture in Shkodra. The pots were held at a moisture equal to 75% of field capacity, based on the method of water field capacity (WFC) (Klute A, 1986), adding distilled water (H_2O) based on gravimetric method as WFC was calculated in advance. Soil pots were checked daily and water loss was replenished by bringing the pots back to weight. Nitrogen use efficiency (NUE) was calculated as above ground biomass dry matter produced per unit of N-fertiliser and N-amendments applied.

Wheat straw was preliminary grinded at <0.2 cm and then applied at a dose of 10 Mg ha^{-1} (on dry weight basis), corresponding to 40 g pot^{-1}, in T3, T5 and T6. Many studies have shown that wheat straw is rich in organic material and soil nutrients (Saroa and Lal, 2003; Tan *et al*., 2007; Lee, 2010) and the addition of crop residues to cultivated soils helps to improve the soil quality and productivity due to its favourable effects on soil properties (Mulumba and Lal, 2008). N concentration in wheat straw is 0.39% or 3.90 g kg^{-1} (Khan *et al*., 2012).

Biochar from wheat straw was applied at a dose of 10 Mg ha^{-1} (on dry weight basis) in T7, from which 40 g of biochar were mixed with 400 g of soil, previously exceeded 2 mm sieve, and then well mixed in 6600 g 5 mm sieved soil in each pot. As a soil amendment, biochar can greatly influence various soil properties and processes (Lehmann and Joseph, 2009). The presence of biochar in the soil can improve soil chemical (*e.g*., pH, CEC) (Liang *et al*., 2006) and physical properties (*e.g*., soil water retention, hydraulic conductivity) (Major *et al*., 2009). Total N concentration in biochar from wheat straw is 4.60 g kg^{-1} based on laboratory results (Singh *et al*., 2017). The main physical and chemical properties of the wheat straw and the biochar used in the experiment are reported in Table 2.

Soil sampling and analysis

Before trial started, a representative soil sample (1 kg) was used to determine physical and chemical characteristics. Soil texture was determined by the pipette gravimetric method. pH was measured on 1:2.5 (w/s) aqueous soil extract. Humidity (W) was established by weighing the soil before and after drying in an oven at 105°C (ISO 11465). Total N was determined after digestion with H_2SO_4 and H_2O_2 according to Kjeldahl method (DIN EN 16169, 2012). Total P was measured according to the ascorbic acid molybdenum blue method (DIN EN 16169, 2012; Murphy and Riley, 1962). NO_3^--N was determined within 24 h, by using KCl 2*M* extraction followed by measurement with spectrophotometer, according to Keeney and Nelson (1982). Organic carbon was measured with the Walkley and Black method (1934), after oxidation of organic matter by potassium dicromate ($K_2Cr_2O_7$) in mixture with H_2SO_4, followed by tritation with ammonium ferrous sulphate [$Fe(NH_4)_2(SO_4)_2 \times 6H_2O$]. The micronutrients, such as Mg, Fe, K, Ca, were analysed with a Flame Atomic Absorption Spectrometer type AA350, according to the Method 3051A (2007).

During the three months of experiment, soil samples were taken three times, at interval of one month, from the pots. They were collected with a sterile probe by plunging in pot 0-10 cm depth and extracting the probe carefully to avoid disturbing the soil structure in the pot. The empty space created after the sampling was filled with a clean plastic pipe to precisely maintain the soil structure. Soil samples were analysed for NO_3^--N and Total N. NO_3^--N -was measured immediately after the sampling. For Total N measurement, the soil samples were air dried, sieved at 2 mm, well mixed and then analysed.

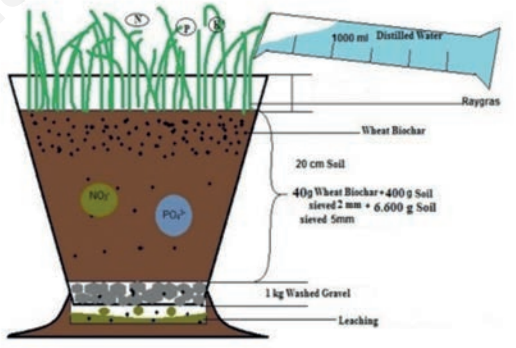

Figure 2. Pot experiment design.

Table 2. Main physical and chemical parameters measured on wheat straw and biochar from wheat straw used in the experiment.

Parameter	Amendment type	
	Biochar	Wheat straw
pH	10.51	-
Organic matter (g kg^{-1})	-	669.15
Total C (%)	48.53	9.83
Total N (%)	0.46	0.37
Total P (%)	0.11	37.81
Total K (%)	5.24	-
Specific surface area (m^2 g^{-1})	4.81	-
Pore volume (cm^3 g^{-1})	0.0051	-
Pore width (nm)	5.00	-

Plant sampling and analysis

One month after planting, the plants were cut at 2-3 cm from the soil surface of each pot and weighed. After cutting, the plants were uniformly washed with distilled water to eliminate adhering substances and dried in an oven at 60°C, till constant weight. Then they were grounded in a Wiley mill and analysed for Total N, after digestion with Kjeldhal method (Taylor and Francis Group, 1998). After one and two months from the first plant sampling, the second and the third cuts were respectively realised for all the treatments.

Leaching water sampling and analysis

After each of the three plant cuts, the soils of each pot were subjected to leaching. This was achieved by filling the pots of each treatment with 1000 mL of distilled water by a very slow process of water application (Figure 3) and expecting the irrigation finished in order to collect the drainage water. The amount of water used for leaching corresponds to a rainfall of almost 26 mm, which happens frequently in the Skodra region. A total of three leaching cycles were applied to the pots, following the same procedure. From each of the seven treatments, a leachate of 450 mL on average was collected. The percolate of each pot was stored in dark plastic bottles in refrigerator at a temperature of 4°C and NO_3^--N was determined within 24 h, according to UNEP/MAP/MED POL (2005).

Statistical analysis

The data collected during the three months of experimental trial were statistically analysed by ANOVA (two tailed). Since the data did not show a normal distribution, the statistically significant differences between means were tested using the nonparametric Kruskal Wallis test (P<0.05).

Results and discussion

Soil properties

The results of physical analysis (Table 3) showed that the soil used in the experiment had a **s**andy loam texture (United States Department of Agriculture classification), with 43% of sand. The chemical analysis showed a moderate amount of macro and microelements in soil composition (Table 3).

Leached NO_3^--N

The values obtained based on NO_3^--N concentration in leachate in mg L^{-1} and the amount of leaching water in mL served to calculate the NO_3^--N in mg kg^{-1} soil leached from each treatment after each plant cut and the NO_3^--N in kg N ha^{-1} leached from the soil surface to the soil depth of 30 cm (Table 4).

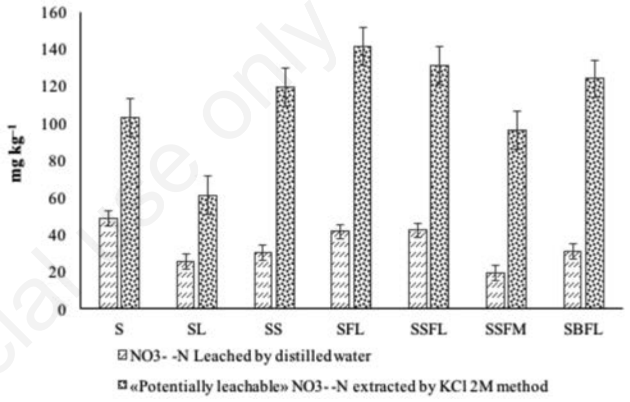

Figure 3. Comparison between the corresponding values of NO_3^--N according to leaching with distilled water and extraction with 2M KCl.

Table 3. Main physical and chemical characteristics of the soil used in the experiment.

Soil type	Physical characteristics					Chemical characteristics							
	Sandy (%)	Silty (%)	Clay (%)	W (%)	pH	NO_3^--N (mg kg^{-1})	Total-N (g kg^{-1})	Total-P (g kg^{-1})	Organic-C (g kg^{-1})	Mg (g kg^{-1})	Fe (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})
SLS	43.19	33.67	23.14	5.08	8.00	166.16	1.560	0.67	19.47	17.29	15.65	4.69	0.80

SLS, sandy loam soil.

Table 4. NO_3^--N leached from the soil, as determined by soil leaching with distilled water and expressed as average value of four replications.

Treatment	Concentration of NO_3^--N in leachate			Leached NO_3^--N				NO_3^--N leached from the 0-30 cm soil layer
	1st cut	2nd cut mg L^{-1}	3rd cut	1st cut	2nd cut mg kg^{-1} soil	3rd cut	Amount mg kg^{-1} soil	kg N ha^{-1}
S	189.58±41.41^{a}	293.15±46.81^{a}	275.27±40.72^{b}	12.19	18.67	17.62	48.48	163.77
SL	139.25±25.70^{b}	134.81±16.35^{b}	133.79±19.12^{c}	8.95	8.85	7.70	25.50	106.04
SS	63.82±16.82^{c}	134.79±53.24^{b}	272.75±50.25^{b}	4.04	8.66	17.53	30.24	108.85
SFL	79.77±16.49^{d}	241.92±20.66 ^{c}	325.28±25.8^{a}	5.13	15.55	20.91	41.59	149.73
SSFL	91.00±15.71^{e}	266.80±32.7 ^{c}	300.00±40.04^{ab}	5.85	17.15	19.29	42.29	156.47
SSFM	96.72±29.82^{b}	95.16±28.85^{d}	105.20±37.59^{c}	6.22	6.14	6.76	19.12	50.83
SBFL	70.49±13.26d ^{c}	64.26±8.27^{e}	112.10±4.16^{c}	8.81	8.03	14.01	30.84	111.04

$^{a-e}$Means followed by same letters in each column are not different (significant differences at P<0.05).

NO_3^--N concentrations in leachate expressed in mg L^{-1} showed an increase from the 1st cut to the 3rd cut in all the considered treatments, except for SL treatment where the NO_3^--N values were pratically constant. In the third cut, maize treatment (SSFM) showed the lowest NO_3^--N concentration (<110 mg L^{-1}). The lower nitrate-nitrogen concentration values of the 1st cut were observed in SS and SBLF treatments, lower or near to 70 mg L^{-1}, while the highest value was obtained in control (S) (189.58 mg L^{-1}). In the 2nd cut, the higher NO_3^--N concentrations were found in S, SSFL and SFL, with 293.15±46.81; 266.8±32.7 and 241.92±20.66 mg L^{-1}, respectively. Particularly, in SFL and SSFL NO_3^--N values were about three times higher compared with the values recorded after the 1st cut in the same treatments. For the control (S) the NO_3^--N value after the 2nd cut was 55% higher than after the 1st one. In SSFM, the NO_3^--N concentration was about 9% lower than the 1st cut. The lowest NO_3^--N value of all the other treatments after the 2nd cut was found in SBFL (64.26±8.27 mg L^{-1}). The NO_3^--N values observed after the 2nd and the 3rd cut were not different for S, SL, and SSFL treatments; while NO_3^--N values after the 3rd cut were two times higher than the 2nd in the case of SS, 80% higher in the case of SBFL and 15% higher in the case of SSFM.

The comparison between NO_3^--N values observed in S and SL both after 2nd and 3th cut showed that in the absence of the root system of plants NO_3^--N leached from bare soil was twice higher than the soil cultivated with ryegrass, confirming once again the undisputable effect of nitrates uptake by plant. The comparison between NO_3^--N leached in the treatments with biochar and wheat straw, in presence of ryegrass and NPK fertilisation (SBFL *vs* SSFL) showed the retention effect of biochar, both during the 3rd and the 2nd cut (112 *vs* 300 and 64 *vs* 267 mg L^{-1}, respectively). When maize was cultivated in presence of wheat straw amendment and NPK fertilisation, an intensive nitrate retention system was defined in all the three cuts compared to all the other treatments, due to the fact that the root system acts as a sink.

Total amount of leached NO_3^--N in mg kg^{-1} soil (Table 4) followed the same trend as in the case of NO_3^--N concentration in leachate. In general, higher values were found in the 3rd cut compared to the 2nd one for all the treatments, except for SL and SSFM where leached NO_3^--N were approximately 7 mg kg^{-1} soil. The same values expressed in kg N ha^{-1} resulted in the following ranking among the different treatments: $S>SSFL \geq SFL>SBFL \geq SS \geq SL>SSFM$. From the results of this experiment, significant findings could be observed: i) ryegrass and wheat straw, with or without NPK, had the same effect on NO_3^--N retention; ii) SL and SS treatments showed a similar amount of NO_3^--N leached from 0.30 cm soil depth, with a reduction of about 35% compared to the control (S); iii) biochar, in presence of NPK fertilisation and ryegrass was more effective in reducing NO_3^--N leaching from 0.30 soil depth than SFL and SSFL treatments. Indeed, in SBLF, NO_3^--N leached was lower of about 27% than SFL (111.04 vs. 156.47 kg N ha^{-1}) and 26% than SSFL (111.04 *vs* 149.73 kg N ha^{-1}); iv) the effect of maize root system (SSFM) on NO_3^--N retention was two and three times higher compared to biochar and heat straw (50.83 *vs* 111.04 and 149.73 kg ha^{-1} NO_3^--N), respectively.

Potentially leachable (PL) NO_3^--N

One of the objectives of the study was to determine the NO_3^--N potentially available to be leached (*PL*) from soil using 2*M* KCl extraction (Bremner, 1965; McTaggart and Smith, 1993). This extraction method was applied to the soil of each experimental treatment after each of the three plant cuts. The obtained results (Table 5) showed that *PL* NO_3^--N was higher compared to NO_3^--N leached by distilled water, and this because the different extraction methods applied (leaching with 1 l of distilled water vs. extraction with 200 mL 2*M* KCl diluted in 100 g of soil). The value of *PL* NO_3^--N expressed in mg kg^{-1} soil was high in all the treatments (Table 5). *PL* NO_3^--N values showed different patterns: a decrease of *PL* NO_3^--N from the 1st to the 3rd cut was registered for the SSFM treatment; highest values after the 2nd cut compared to the 1st and the 3rd cut for SL and SSFL treatments; increasing values from the 1st to the 3rd cut for all the other treatments.

The cumulative *PL* NO_3^--N values were from 60.76 (SL) to 141.10 mg kg^{-1} soil (SFL). *PL* NO_3^--N was 40% lower in SL compared to S (60.76 *vs* 102.67 kg N ha^{-1}). On average, the *PL* NO_3^--N values founded in straw treatments (SS and SSFL) were higher compared to the bare soil of about 22%. We presume that these differences come as a result of straw mineralisation process occurred during the 14 weaks of greenhouse conditions. In SS treatment, the sharp increase of *PL* NO_3^--N values observed from after the 1st to the 3rd cut should be due to the progressive mineralisation process of straw (7.22, 44.77 and 67.15 mg kg^{-1} soil per 1st, 2nd and 3rd cuts, respectively). As for NO_3^--N leached by distilled water also for *PL* NO_3^--N the cumulative values were higher in SFL and SSFL (141.1 and 130.9 mg kg^{-1} soil, respectively). For SBFL the cumulative *PL* NO_3^--N values were lower compared to SFL and SSFL. The effect of biochar on NO_3^--N retention, as also discussed in previous studies (Libutti *et al*., 2016), should be due to the improved cation exchange capacity (Glaser *et al*., 2002; Cornelissen *et al*., 2013) and improvement of soil physical and hydraulic properties (Bruun *et al*., 2014).

Table 5. ***Potentially leachable*** **NO_3^--N (*PL* NO_3^--N) in the soil, as determined by soil extraction with *2M* KCl and expressed as average value of four replications.**

Treatment	*PL* NO_3^--N (mg kg^{-1} soil) 1st cut	2nd cut	3rd cut	Amount mg kg^{-1} soil	kg N ha^{-1}	*PL* NO_3^--N/Total -N (%)
S	24.09 ± 0.78^{b}	28.24 ± 1.84^{bc}	50.34 ± 1.33^{b}	102.67	369.60	6.58
SL	17.70 ± 0.33^{c}	24.98 ± 2.13^{c}	18.06 ± 0.99^{c}	60.76	218.75	3.89
SS	7.22 ± 0.43^{d}	44.77 ± 4.36^{b}	67.15 ± 0.99^{a}	119.14	428.89	7.64
SFL	22.32 ± 3.21^{bc}	59.29 ± 3.96^{ab}	59.49 ± 1.48^{ab}	141.10	507.98	9.04
SSFL	24.22 ± 3.41^{b}	64.80 ± 2.58^{a}	41.86 ± 1.87^{bc}	130.87	471.15	8.93
SSFM	45.47 ± 1.93^{a}	30.10 ± 1.31^{bc}	20.09 ± 2.25^{c}	95.70	344.53	4.46
SBFL	22.20 ± 4.50^{bc}	45.34±2.45b	56.22 ± 1.36^{ab}	123.77	445.56	7.93

[a-d]Means followed by same letters in each column are not different (significant differences at $P<0.05$).

The amount of *PL* NO_3^--N calculated per hectar of soil (Table 5) was quite high in all the treatments, showing a range from 219 (SL) to 508 kg ha^{-1} (SFL). Expressed as percentage of the total N in the bare soil before trial started (1560 mg kg^{-1} soil; Table 3), *PL* NO_3^--N showed a high variability; from 3.89% in treatment with ryegrass without NPK (SL) and up to 9.04% in treatment with ryegrass and with NPK (SFL). If we compare the first treatment (S) and the second one (SL), we can notice that the presence of ryegrass has contributed to decrease by half the amount of *PL* NO_3^--N. In the treatments where wheat straw, mineral nitrogen fertiliser and ryegrass were applied (SFL and SSFL), the percentage of mineralised nitrogen was higher than in the case of bare soil. Grazhdani *et al*., (1996) in a similar type of soil (total nitrogen content of 1870 mg kg^{-1} soil and loamy sandy soil texture) measured in an incubation test experiment of 33 weeks the *N potentially mineralisable pool* and founded it was 13% of the total organic nitrogen stock, or twice the nitrogen extracted on bare soils.

The cumulative NO_3^--N values by the two methods (extraction with 2*M* KCl and leaching with distilled water) are showed in Figure 3. In the first two treatments (S and SL, respectively), the ratio between *PL* NO_3^--N and leached NO_3^--N was close to 2 (2.1 and 2.3 for S and SL, respectively). The KCl extraction method extracted at least the double amount of nitrate compared to distilled water in all the treatments. In SS treatment, this ratio was about four times higher due to enhanced microbial activity from the presence of straw that should has also increased the mineralisation of organic N in the soil as well as organic N in the straw. In fertilised and wheat straw amended treatments (SFL and SSFL) this ratio was higher than 3. The presence of biochar, due to its retention capacity of mineral N, increased the ratio between *PL* NO_3^--N and leached NO_3^--N to 4. The maximum value of the ratio was observed in SFLM, although the *PL* NO_3^--N and NO_3^--N values were relatively low (95.7 and 19.12 mg kg^{-1} soil, respectively) compared with the other fertilised and organic amended treatments, due to the important role of maize root system in N uptake. Mineralisation of the organic amendments and fertiliser used were sharply increased by a more intensive microbial activity, enhanced from the presence of the root system.

Nitrogen plant uptake and apparent nitrogen use efficiency

The nitrogen amounts absorbed by plant showed differences between cuts and treatments (Table 6). In the 1st cut, N uptake values expressed in mg pot^{-1} were higher than the other two cuts and varied from 40 in SL up to 187 in SSFM. The other three treatments did not show statistically significant differences among them. In the 3rd cut for all the treatments with fertiliser and/or organic amendments, the values of N uptake were lower or at least equal than the previous two cuts. As expected, in all the three cuts, the values of N uptake were lower in non-fertilised treatment compared to the nitrogen fertiliser treatments. There were no differences in N uptake values between SSFL and SBFL (339.8 *vs* 350.4 mg pot^{-1}, respectively), and in the presence of maize this value was higher (406.5 mg pot^{-1}) than the ryegrass.

The total N uptake values in mg kg^{-1} soil for the three cuts, ranged from 18.16 in non-fertilised treatment (SL) to 58.06 for SSFM. In SSFL, N uptake value was lower than SFL, showing an inhibitory effect of organic substrate in absorbing N from fertiliser. The organic substrates (SSFL and SBFL) effect in absorbing nitrogen from the soil was comparable. This appears also in the values of N uptake expressed in mg kg^{-1} soil and kg N ha^{-1}, and nitrogen use efficiency. Maize (SSFM treatment) showed higher N uptake ability and NUE compared to ryegrass (SL, SFl, SSFl and SBFL treatments) (N total uptake and NUE were 209 kg N ha^{-1} and 47.8%, respectively). The lowest value of NUE was observed in treatment with nitrogen fertilised and wheat straw (SSFL, close to 36.5%) while for biochar treatment NUE was slightly higher but not significantly different ($P<0.01$) (36.46 *vs* 38.27%, respectively). This result highlights the effectiveness of biochar soil amendment not only in nitrate retention but also in plant nutrition (Kammann *et al.*, 2011).

Conclusions

A three months pot experiment, carried out under greenhouse conditions with *Lolium multiflorum* L. and *Zea mays* L., was aimed at evaluate the effect of wheat straw and biochar amendment on nitrate retention of a sandy loam soil, under the same rate of NPK fertiliser.

The main findings observed in the course of the study are: i) biochar reduced of about 26% the amount of NO_3^--N leached from 0.30 cm soil depth compared to wheat straw, in presence of NPK fertilisation and ryegrass; ii) the effect of maize root system on NO_3^--N leaching reduction was two and three times higher compared to biochar and wheat straw, respectively. Furthemore, the amount of *PL* NO_3^--N calculated per hectar of soil was higher in the treatments where wheat straw, mineral nitrogen fertiliser and ryegrass were applied. To the amount of extracted nitrogen contributed also nitrogen added by wheat straw mineralisation and fertiliser. Results from the study indicated a mitigating action of biochar on leaching of NO_3^--N. In presence of plant roots and fertilisation, this effect was higher than wheat straw. However, being a short-term experiment, future research needs to test the same biochar and wheat straw application on soil in long-term experimental trials under field conditions.

Table 6. Nitrogen plant uptake and nitrogen use efficiency.

Treatment	N uptake (mg pot^{-1}) 1st cut	2nd cut	3rd cut	Amount	Total N uptake mg kg^{-1} soil	kg N ha^{-1}	Nitrogen use efficiency (%)
SL	39.99 ± 5.55^{c}	42.12 ± 2.46^{c}	45.21 ± 4.32^{c}	127.32	18.16	65.37	0
SFL	161.8 ± 6.34^{b}	127.5 ± 6.87^{a}	97.3 ± 10.63^{a}	386.6	55.23	198.82	44.48
SSFL	152.5 ± 6.81^{ab}	92.7 ± 11.79^{b}	94.6 ± 14.16^{ab}	339.8	48.54	174.75	36.46
SSFM	187.1 ± 1.76^{a}	127.9 ± 3.42^{a}	91.5 ± 16.44^{ab}	406.5	58.06	209.01	47.87
SBFL	154.3 ± 5.23^{ab}	107.2 ± 17.34^{ab}	88.9 ± 7.76^{b}	350.4	50.04	180.21	38.27

$^{a-c}$Means followed by same letters in each column are not different (significant differences at $P<0.05$).

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