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Risk of nitrogen losses from cultivated soils: effect of biochar amendment

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Abstract

Field experiments were carried out in Southern Italy (Apulia Region) to evaluate the effect of biochar amendment on nitrogen (N) potential losses from the soil. Three vegetable crops were grown in close succession on a clay loam soil: chicory (*Chicorium intybus*, L.), processing tomato (*Lycopersicon esculentum*, L.) and lettuce (*Lactuca sativa*, L.). N fertilization was performed according to the local standard practices. Biochar obtained from wood chips was mixed within the upper 0.20 m soil layer. Seven treatments were compared: B_0, no biochar addition (control); B_{10}, and B_{20}, a single and a double biochar dose (10 and 20 t ha^{-1}, respectively), both applied only once; B_{10}^+ and B_{20}^+, similar to the previous treatments but with biochar applied twice; B_0-$N_{1/2}$ and B_{20}-$N_{1/2}$, like B_0, and B_{20}, respectively, but providing half of the N supply. The apparent soil N balance was estimated considering the topsoil (0.20 m). N balance was affected by N fertilization and biochar application. When N fertilization rate was halved ($N_{1/2}$), the N apparent deficit was significantly reduced, particularly in tomato (119 kg ha^{-1} of N), but also in chicory and lettuce crops (55 and 64 kg ha^{-1} of N, respectively). Similarly, biochar application (both B_{10} and B_{20}) significantly reduced N apparent deficit in the three crops, as compared to B_0. Considering the highest biochar rate (B_{20}), again a higher effect was detected in tomato (84 kg ha^{-1} of N), than in chicory and lettuce crops (39 and 41 kg ha^{-1} of N, respectively). The experimental outcomes showed that biochar application to cultivated soils could be an effective option in mitigating N losses, particularly in highly risky conditions of unbalanced N supply.

Keywords: biochar, N fertilization, N uptake, soil N balance, soil N loss, vegetable crops.

1. Introduction

Nitrogen (N) is an essential element for plant growth and development which significantly increases the agricultural yield and its quality. Over the last half century, the application of N inorganic fertilizers had a very large effect on soil productivity and crop yields and allowed meeting the needs of a growing human population for food. However, inorganic N inputs do not always ensure high yields. Due to the dynamic nature of this nutrient, significant fractions of N can be lost from soil-plant systems via leaching, surface runoff, denitrification and volatilization, causing a lowering of plant-available N and a reduction of crop yields [22]. N losses can also lead to numerous negative impacts on the environment, such as excess nitrate (NO_3^-) in groundwater, eutrophication of water bodies and nitrous oxide (N_2O) emission in the atmosphere [47]. Therefore, N-use efficiency needs to be maximized to improve the economic sustainability of cropping systems [17] and maintain environmental quality [7].

Previous studies have shown that adding organic residues to the soil preserves soil organic carbon and fertility [37], provides a source of labile carbon to the soil microorganisms involved in N transformations [43] and is an important tool in retaining N in the soil [4]. In recent years, biochar has emerged as a promising soil additive to reduce N losses from cultivated soils and improve N-use efficiency for a sustainable crop production [27]. Biochar is a carbon-rich compound obtained by heating agricultural biomasses in the complete or near absence of oxygen (pyrolysis or gasification) and provides a useful amendment for the improvement of soil quality [29, 36, 50, 51], whilst sequestering atmospheric CO_2 and reducing greenhouse gas emissions [26]. A number of beneficial effects have been documented when biochar is applied to soil, including improved soil organic carbon [18], water holding capacity, soil structure and porosity [1], increased cation and anion exchange capacity [30], liming effect [49], decreased soil bulk density [20], increased nutrient retention and availability, decreased nutrient leaching and fertilizer needs [28]. Biochar also stimulates soil microbial biomass and activity [44], increases soil fertility, enhances plant growth and crop productivity [34].

Several studies showed that biochar affects N cycling, by reducing inorganic N leaching [40], ammonia volatilization [42] and N_2O emissions [41], while also increasing biological N fixation [39]. In a multilayered soil column experiment, where bamboo biochar was mixed to the first 0.20 m soil depth, cumulative losses of ammonium-nitrogen (NH_4-N) via leaching were reduced by 15.2 % [15]. Another leaching column experiment [48] showed a reduction of NH_4-N by 14%, after addition to the soil of peanut hull biochar at a high rate (2 % of the soil, w/w). Also [14], found a NH_4^+ leaching reduction when biochar was added to a sandy soil. A number of studies carried out in pot, leaching column, laboratory and open field trials [23, 48, 11, 19, 9, 6, 24, 31, 13] showed decreases in leaching of NO_3^- after soil amendment with biochar. Nevertheless, the effect of biochar on soil N retention depends on the type of soil, type of biochar and level of biochar use [5] and the evidence that biochar amendment increases soil N retention in the field is also contradictory [24]. Other authors found increases in the concentration of NH_4-N and NO_3-N in soil solution and a significant N nutrient leaching after the application of wood biochar to the soil [35]. Similarly, other experiments [48, 21, 16] showed the poor ability or inability of biochar to retain NO_3-. Therefore, the effect of biochar on N retention and leaching must be studied in soil-biochar-plant systems, particularly when soils are heavily fertilized.

The aim of this study was to investigate the effect of biochar amendment on the risk of N losses from an intensively cultivated soil, in Southern Italy (Apulia Region). More specifically, three rates of biochar application to the upper 0.20 soil layer, two frequencies of biochar soil addition and two rates of N fertilizers supply to the plants were evaluated within a close succession of three vegetables crop, such as chicory (*Chicorium intybus*, L.), processing tomato (*Lycopersicon esculentum*, L.) and lettuce (*Lactuca sativa*, L.). The specific objective was to assess the ability of biochar to retain N in the soil, thus mitigating N losses from the soil-plant system and avoiding or reducing the impacts of N soil losses on the sustainability of cropping systems and the environmental quality.

2. Material and Methods

2.1. Site description and agronomic conditions

The trials were carried out in open field, from September 2014 to January 2016, in an agricultural area of the Apulian Region (Southern Italy), at Lucera (41°30’19’’n, 15°20’20’’E, 217 m a.s.l.), in the Foggia district. The area is characterized by a Mediterranean climate, with air temperatures that drops below 0 °C in winter and exceed peaks of 40 °C in summer. The long-term average annual rainfall is 590 mm, with precipitations unevenly distributed throughout the year and predominantly concentrated in the period from October to April [10].

Three crops were grown in close succession: chicory (*Chicorium intybus*, L.), processing tomato (*Lycopersicon esculentum*, L.) and lettuce (*Lactuca sativa*, L.), respectively. Chicory (cv. Catalogna) was transplanted on December 12, 2014; processing tomato (cv. PS21) on April 15, 2015; lettuce (cv. Mortarella d’inverno) on October 19, 2015. Seedlings were placed in double rows (50 cm apart) spaced at 190 cm, at a distance of 30 cm along each single row, reaching a final plant density of 3.5 plants m^{-2}. The entire plants (shoots) of chicory, tomato and lettuce were harvested at the full maturity stage after 137, 132, 89 days from transplanting, respectively.

Irrigation scheduling was performed according to the evapotranspiration criterion with watering’s carried out every time the total available soil moisture was depleted to the threshold value of 50%. The reference evapotranspiration was calculated daily according to the FAO Penman-Monteith equation [2]. For each considered crop, the maximum crop evapotranspiration (ETc) was estimated daily according to the classical ‘two-step’ procedure, i.e. by multiplying the reference evapotranspiration by the crop coefficients as proposed by the FAO Irrigation and Drainage Paper N. 56 [2]. To calculate the daily reference evapotranspiration and to daily update the water balance, the considered meteorological variables were: maximum and minimum air temperature (T_{MAX} and T_{MIN}, °C) and humidity (R_{MAX} and R_{MIN}, %), wind speed (W_S, m s^{-1}), and total precipitation (P, mm). These were measured by a weather station placed close to the experimental field and recorded using a data-logger (Campbell Scientific, USA).

Each watering restored the full ETc losses, with the soil water content taken back to field capacity. The seasonal irrigation volume was 4,937 m^3 ha^{-1} for tomato crop, with water volumes varying from 140 to 400 m^3 ha^{-1}, depending on the crop growth stage. The autumn-winter precipitation completely satisfied the ETc of chicory and lettuce. A drip irrigation method was used. This comprised–a single drip line, with drippers at 2 l h^{-1} flow rate, spaced every 40 cm, and placed in the middle of each couple of plant rows.

All agricultural management practices, including fertilization, weed and pest control applied to the three crops, were performed according to the agronomic techniques commonly adopted by local farmers. Pre-transplanting fertilizations were applied to chicory, tomato and lettuce, by distributing 42 kg ha^{-1} N and 60 kg ha^{-1} P_2O_5; 98 kg ha^{-1} N and 56 kg ha^{-1} P_2O_5; 60 kg ha^{-1} N and 36 kg ha^{-1} P_2O_5, respectively. A top-dressing fertilization was also applied by distributing 40, 104, 52 kg ha^{-1} N to chicory, tomato and lettuce, respectively.

The trials were carried out on a clay loam soil (United States Department of Agriculture classification), with a field capacity (-0.03 MPa) of 29.20 % dry weight (dw), a wilting point (-1.5 MPa) of 17.10 % dw, and a bulk density of 1.20 Mg m^{-3}. The main soil characteristics (0-60 cm) are as follows: sand, 28.00 %; loam 40.20 %; clay 31.80 %; organic matter 1.64%; Olsen P_2O_5, 92.10 mg kg^{-1}; total N (Kjeldahl), 1.20‰; mineral NO_3-N, 9.60 mg kg^{-1}; mineral NH_4-N, 19.60 mg kg^{-1}; pH 7.79; electrical conductivity, 0.60 dS m^{-1}.

2.2. Biochar

The biochar used in the experiments was obtained by pyrogasification of fire wood chips at temperature of 1,200°C, in a 200 kWe gasifies unit. Before trials started, biochar was analyzed for a set of physico-chemical characteristics. Proximate analysis, using a TGA analyzer unit (LECO-TGA701), according to the ASTM D7582 method, was applied in order to determine the biochar content in total solid (TS), volatile solid (VS), ash (AS) and fixed carbon (FC). Ultimate analysis, using a CHNS Elemental Analyzer (CHN LECO 680), according to the method LECO-ASTM D5373, determined the C, N, H and S biochar content. O content was calculated by the difference: O (%) = 100-C-H-N-S-ash. From H, C and O content, the molar ratios of hydrogen to organic carbon (H/C_{org}) and oxygen to organic carbon (O/C_{org}) were obtained. pH and electrical conductivity were determined in water extract by a GLP 22+ pH-meter and a GLP 31+ EC-meter (Crison Instruments, Barcelona), respectively, using a biochar to deionized water mass ratio 1:20, followed by shaking and waiting an equilibrium time of 5 min before extracting. Micro- and macro-elements were determined by digesting 0.25 g of sample in 10 ml of HNO_3 in a closed vessel microwave digester (CEM-Mars6) for 20 min at 220 °C and analyzing the metals in the solution by inductively coupling plasma spectrometry-optical emission spectroscopy (ICP-OES Agilent 720). The ring knife method [45] was used to measure bulk density, water-filled porosity, total porosity and air space of the media. The water holding capacity (WHC) was determined applying an adapted protocol from [3].

2.3. Experimental layout

Seven experimental treatments, each one replicated three time, were arranged in the field according to a randomized block design, for a total of 21 plots. Each plot was 64 m^2 (8 m wide x 8 m long). In particular, the effects of three amounts of biochar in the soil, two frequencies of biochar addition to soil and two nitrogen fertilization rates were evaluated. The applied treatments were as follows: B_0, no biochar addition (control); B_{10}, one biochar dose (10 t ha^{-1}) applied only once (on September 2014); B_{20}, double biochar dose (20 t ha^{-1}) applied only once (on September 2014); B_{10}^{+}, one biochar dose, but applied twice (on September 2014 and September 2015); B_{20}^{+}, double biochar dose, but applied twice (on September 2014 and September 2015); B_0-$N_{1/2}$, like B_0, but considering half of the N supply to each crops; B_{20}-$N_{1/2}$, like B_{20}, but considering half of the N supply to each crop. Before the establishment of the experiments, the soil was harrowed to a depth of 40 cm. Then, the different biochar rates were respectively applied to the surface of each experimental plot and homogeneously mixed to the soil within the upper 0.20 m soil layer, through a milling operation.

2.4. Soil sampling and analysis

Soil samples were collected in triplicate from each plots, at the start and the end of each crop cycle. Each replicate consisted of a soil core sampled within the upper 20 cm of depths, using a 50-mm-diameter soil auger. Therefore, 63 samples in total were obtained every sampling date. Samples were stored and maintained frozen before determining the NO_3-N and the NH_4-N concentration by soil extraction with 2 M KCl, followed by spectrophotometric analysis of the extract [25].

Before trial started, a soil physico-chemical characterization was carried out. Soil samples were air-dried, crushed, passed through a 2-mm sieve and analyzed. The particle-size distribution was determined using the pipette-gravimetric method. The water holding capacities at -0.03 MPa and -1.5 MPa were obtained using a pressure-plate apparatus (Soilmoisture Equipment Corp.), with their difference providing the maximum crop-available water. The bulk density was measured by extracting three intact soil cores at 20, 40, and 60 cm soil depths, with an 'undisturbed' soil-core sampler (Model 0200, Soilmoisture Equipment Corp). The pH and electrical conductivity were measured on 1:2.5 (w/v) aqueous soil extracts and saturated soil paste extracts respectively. The available phosphorus was determined by the sodium bicarbonate method [38], and the total organic carbon by the acid dichromate digestion technique [46]. The total N was obtained according to the Kjeldahl method [8]. NO_3-N NH_4-N were determined as abovementioned.

2.5 Plant sampling and analysis

Chicory, tomato and lettuce plants were sampled at the harvesting date, in triplicate from a sampling area of 20 m^2 in each experimental plot. Tomato fruit samples were similarly collected, by picking all of the mature fruit. The plants and fruits were transported immediately to the laboratory and analyzed for NO_3-N and the NH_4-N content, by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA).

2.6 Experimental data processing and statistical analysis

An N balance was performed comparing the amount of N in a considered soil layer at the end (N_{end}) and at the start (N_{start}) of each crop cycle. It should be specified that these N values are stocks, i.e. state variables related to the soil as N reservoir. Of course, state conditions are dynamically linked to N flows, i.e. N rate variables accounting for amounts of N added to (N_{input}) and removed from the soil-cropping system or even lost (N_{output}) in the course of the three consecutive cropping seasons. The following general balance equation can be drawn:

$$\Delta N = N_{end} - N_{start} = N_{input} - N_{output} \quad (1)$$

A positive value of N balance ($\Delta N>0$) suggests that N is gained by the system (N surplus), while a negative value ($\Delta N<0$) that N losses are prevailing (N deficit). This apparent N balance estimates the potential loss of N from agricultural land; indeed, if a large deficit is occurring, this is suggesting a risk of N losses, potentially causing soil, water and air pollution. The higher is the estimated deficit, the higher is the risk of an undesirable environmental impact.

Considering the balance equation (1), it is worth to detail that the terms of balance are expressed as amounts of N per unit of agricultural area (kg N ha^{-1}). Moreover, the N balance was performed only considering the topsoil, i.e. the first 0.20 m of soil layer. This soil layer is specifically the depth involved in biochar applications to soil. The focus of the trials, indeed, is to compare the effect of biochar addition to soil with respect to control (no biochar application). Anyhow, also the fertilizer applications were performed within the same soil depth. Finally, in the experiment, only the N-forms directly available to plant uptake were taken into account, such as nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N). It was assumed, indeed, that the total N amount in the soil (both organic and mineral), i.e. the one obtained through a Kjeldahl oxidation procedure, should be linked to the soil reservoir (as state variable), while the focus of the experiment is on N flows that are better expressed by the soluble and mobile N forms, such as NH_4-N and NO_3^-N. A strong advantage when considering N flows instead of N state variables is that the lower N quantities involved in the experiment can significantly reduce the standard error of the estimated values and, therefore, increase the precision and the discriminating power of the applied statistical tools.

Therefore, the N flow terms of the balance equation can be further specified according to the following indications.

N inputs rate (kg N ha^{-1} per crop growing season):

- **N_F = N fertilizer application rate;**
- **N_W = N added through irrigation water and rainfalls;**
- **N_D = N deposited on the soil from the atmosphere;**
- **N_B = N due to biological fixation of atmospheric nitrogen;**
- **N_M = N due to biological mineralization of soil organic matter;**

N outputs rate (kg N ha^{-1} per crop growing season):

- **N_C = N crop uptake during the growing season (considering the total shoot biomass);**
- **N_L = N losses by leaching through deep water percolation;**
- **N_V = N losses by volatilization and denitrification into the atmosphere.**

When two treatments are directly compared and the same conditions have been applied to the experiment with the sole exception of the experimental treatment itself, some of the N balance terms are exactly the same (such as N_W, and N_D), therefore, they can be deleted from the balance equation. Other terms can be experimentally measured (N_F and N_C, together to N_{end} and N_{start}) and the remaining terms (N_B, N_M, N_L, N_V) can be estimated as residual difference from the previous ones. Moreover, all these residual N flow terms are mostly related to environmental, physical, biotic and merobiotic soil conditions and are only indirectly affected by agricultural management (apart the biochar and nitrogen applications); conversely, N_F and N_C are controlled by the agricultural management adopted by the farmer. This considered, it is possible to proceed in grouping homogeneous terms as follows:

- **$\Delta N_{ENV} = N_B + N_M - N_L - N_V$;**
- **$\Delta N_{MNG} = N_F - N_C$;**
- **$\Delta N_{SOIL\ STATE} = N_{end} - N_{start}$**

Therefore, the N balance equation (1) can be rearranged as:

$$\Delta N_{ENV} = \Delta N_{SOIL\ STATE} - \Delta N_{MNG} \qquad (2)$$

Largely negative values of ΔN_{ENV} are pointing out that the cropping system is losing N thus impacting the environment. Largely positive values suggest that a N surplus occurs in the soil. This N amount is still available for the following crop, but also susceptible to be lost in the environment before the following cropping cycle begins. Therefore, only a N balance close to zero indicates an efficient N use.

This considered, the final aim of the present study was to assess if biochar application to agricultural soil can significantly affect the value of ΔN_{ENV} and prevent or reduce N losses. When added to soil, biochar effect in mitigating potential N loss risks would be significant if it could contribute in shifting the N balance from negative values to values approaching zero.

The analysis of variance (ANOVA) was performed according to the applied experimental field design and systematic tests of contrast between single or grouped pairs of treatments were performed, in order to statistically assess the effects of biochar and N application rates on ΔN_{ENV}.

3. Results and Discussion

3.1. Meteorological conditions

The average monthly and seasonal values of the meteorological parameters recorded during the experimental period are reported in Table 1.

During the growing seasons of chicory, mean minimum and maximum air temperatures reached values of 2 and 6 °C, respectively. Very low minimum air temperatures (even below 0 °C) were recorded in this period. Particularly in December and January, extremely cold weather conditions, which also led to snow precipitations, were detected. These conditions highly affected plant growth and development which were strongly slowed. Rainfall was equal to 227 mm, with 39% of total precipitation occurred in January.

The spring-summer crop cycle of tomato was characterized by mean minimum and maximum air temperatures which reached values of 11 and 36°C, respectively. Rainfall was equal to 118 mm with 54% of total precipitation recorded in the first part of cultivation cycle, in June.

Table 1. Main climatic parameters recorded during the chicory, tomato and lettuce growing

Growing season	**Chicory**						**Tomato**						**Lettuce**				
Month	Dec	Jan	Feb	Mar	Apr	**Seasonal value**	May	Jun	Jul	Aug	Sept	**Seasonal value**	Oct	Nov	Dec	Jan	**Seasonal value**
Climatic parameter [a]																	
T_{MAX} (°C)	12.5	12.4	11.8	14.0	18.9	**13.9**	26.2	29.1	35.7	32.4	32.9	**31.3**	17.8	18.1	15.2	14.4	**16.4**
T_{MIN} (°C)	2.2	2.3	2.5	4.5	5.7	**3.4**	11.0	14.8	19.6	19.3	20.7	**17.1**	10.5	7.0	3.8	3.0	**6.1**
R_{MAX} (%)	97.8	96.6	98.8	97.8	96.0	**97.4**	94.6	91.5	75.4	90.8	74.6	**85.4**	98.5	94.8	96.2	95.9	**96.3**
R_{MIN} (%)	60.0	60.1	60.8	63.4	43.6	**57.6**	36.5	35.1	26.5	39.2	32.4	**33.9**	70.7	56.2	59.6	59.7	**61.6**
W_S (m s^{-1})	2.2	2.5	2.2	2.9	2.5	**2.5**	1.8	2.1	1.6	1.4	1.6	**1.7**	0.8	0.7	0.4	0.3	**0.6**
P (mm)	16.8	88.4	38.8	62.8	20.0	**226.8**	26.0	64.2	10.0	17.6	0.4	**118.2**	41.6	37.1	2.0	8.4	**89.1**

[a] TMAX, TMIN, maximum and minimum average air temperature; RMAX, RMIN, maximum and minimum average relative air humidity; WS, average wind speed; P, total precipitation

During the lettuce crop cycle, mean minimum and maximum air temperatures reached values of 3 and 18 °C, respectively. Rainfall was equal to 89 mm, with a peak in October when 47% of total precipitation occurred.

3.2 Biochar

Biochar used in the study was alkaline with a pH of 9.4 and showed a salinity level of 300 m Sm^{-1}.

Proximate analysis indicated that biochar contained 88.0% FC, 8.7% VS and 3.3% AS. Ultimate analysis showed that biochar was carbon-rich, with a C content of 83.2% by mass, and 1.7% H, 0.4% N, 0.05% S and 11.4% O by mass. H/C_{org} and O/C_{org} were equal to 0.25 and 0.10, respectively (lower values of these ratios are correlated with greater carbon stability).

ICP-OES analysis revealed the presence of inorganic nutrients such as K (25244.8 mg kg^{-1}), Ca (4392.5 mg kg^{-1}), Mg (1043.5 mg kg^{-1}) and Fe (1023.6 mg kg^{-1}).

Heavy metals concentrations ranged from not detectable amount for Cd, Co and Pb to amounts as high as 286.6 mg kg^{-1} for Mn.

Physical analyses of biochar showed a bulk density of 0.2 g cm^{-3}, a total porosity of 76.3%, with an air space and a water filled porosity of 5.2 and 71.1% respectively, and a WHC equal to 414.5%.

According to the technical specifications published by the Italian Ministry of Agriculture which approved the inclusion of biochar in the list of soil amendments allowed in the Italian agriculture (Italian Official Journal - General Series No 186, 12-8-2015), biochar is assigned to a "class" on the basis of its percentage content of organic C and ash. Biochar used in our experiments belonged to the Class 1 (C_{org} >60%; ash <10%). Moreover, all the requirements of the Italian technical specifications (salinity ≤1000 mSm^{-1}, pH = 4-12, H/C_{org} ≤0.7) were fully complied.

3.3 Nitrogen balance

The results of the ANOVA performed on the ΔN_{ENV} data (total number = 63: i.e. 7 treatments, 3 cropping cycles, 3 replications) are reported in Table 2.

The outcome of the ANOVA model was highly significant (P<1%), as well as the coefficient of determination (R^2 =0.88). The overall RMSE was equal to 19.8 kg N ha^{-1}. The two single ANOVA factors (biochar treatment and cropping cycle) and their reciprocal interaction came out to be statistically significant (P<1%). Therefore, the data presentation and discussion will be focused on the interaction averages.

Table 2. Results of the analysis of variance (ANOVA). For each factor (biochar and cropping cycle) and factors interaction biochar * cropping cycle), F-Ratio and Probability are reported.

Source	Degree of Freedom	Sum of Square	F ratio	Prob > F	§
Replication	2	2,971.76	3.7963	0.0309	*
A- Biochar/Nitrogen	6	44452.90	18.9291	<.0001	**
B- Cropping Cycle	2	50,034.99	63.9183	<.0001	**
A * B	12	13,914.26	2.9625	0.0049	**
Model	22	111,373.91	12.9343	<.0001	**
Error	40	15,655.93			
Total	62	127,029.84			

§ ** = statistically significant at 1% level; * = statistically significant at 5% level.

Figure 1 shows the mean values of ΔN_{ENV} (kg N ha^{-1}) with respect to the three crops and considering both the N fertilizer application rates to the plants (Fig. 1.A) and the biochar addition rates to the soil (Fig. 1.B).

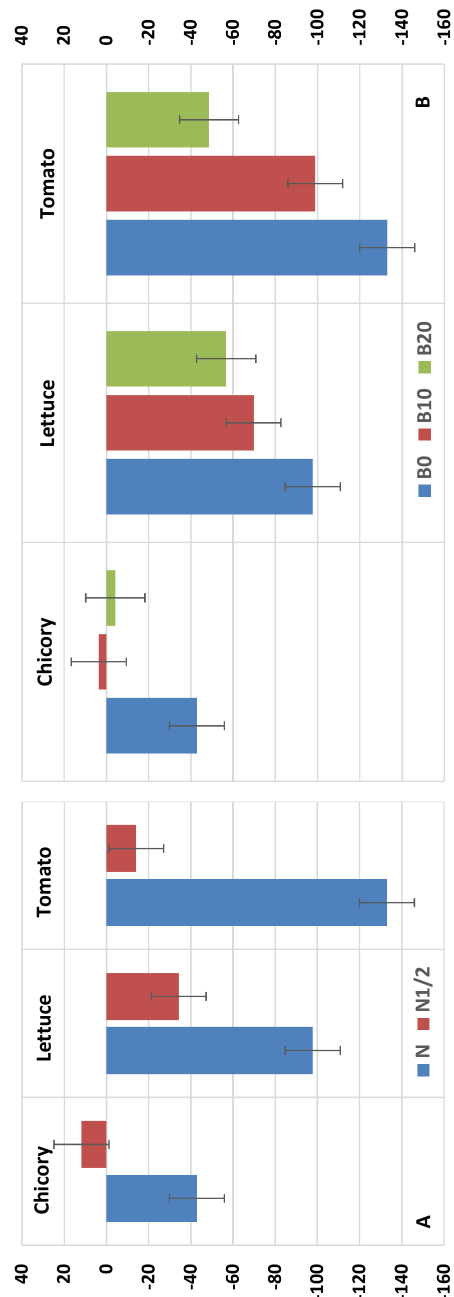

Figure 1. Mean values of ΔN_{ENV} (kg N ha^{-1}) during the three crop cycles, with respect to the N fertilization (Fig. 1.A) and the biochar (Fig. 1.B) application rates.

In this regard, Table 3 reports the effects of N applied to crops and biochar added to soil, in terms of statistical pairs of contrasts for each cultivated species. The contrasts are ranked according to descending values of the sum of square accounted for by each of them. The largest influential effect was showed by the N fertilization rate (Tab. 3, contrast 1: N vs. $N_{1/2}$). Indeed, when the rate of N fertilization was halved ($N_{1/2}$), the N deficit was significantly reduced, particularly in tomato (119 kg N ha^{-1}), but also in chicory and lettuce (55 and 64 kg N ha^{-1}, respectively).

Table 3. Effect of N fertilizer and biochar application rates, in terms of statistical pairs of contrasts for each crop cycle.

Contrast	**Crop 1: Chicory**	**Crop 2: Tomato**	**Crop 3: Lettuce**	**Std Error**	**Sum of Square** §
1. N Vs. $N_{1/2}$	54.7**	118.8**	63.5**	14.0	42,272**
2. B_0 Vs. B_{20}	38.7**	84.3**	41.1**	14.0	25,586**
3. B_0 Vs. B_{10}	46.5**	34.1 *	28.1 *	14.0	8,227**
4. (= 3-2): B_{10} Vs. B_{20}	7.8 $^{n.s.}$	-50.2**	-13.0 $^{n.s.}$	11.4	8,266**
5. $N_{1/2}$ fixed: B_0 Vs. B_{20}	-0.2 $^{n.s.}$	-14.9 $^{n.s.}$	2.7 $^{n.s.}$	16.2	344 $^{n.s.}$
6. B_{20} Vs. B_{20}^{+}	=	=	-9.5 $^{n.s.}$	16.2	134 $^{n.s.}$
7. B_{10} Vs. B_{10}^{+}	=	=	0.8 $^{n.s.}$	16.2	1 $^{n.s.}$

§ ** = statistically significant at 1% level; * = statistically significant at 5% level; $^{n.s.}$ not significant.

Biochar integration into the topsoil was also very influential and significantly reduced N deficit. The highest biochar amount (B_{20}) had a strong effect (P<1%) in reducing N surplus (Tab. 3, contrast 2) in all the three crops but, again, a higher effect was showed in tomato crop (84 kg ha^{-1} of N), than in chicory and lettuce (39 and 41 kg ha^{-1} of N, respectively).

The lower biochar amount (B_{10}), although being effective in preventing N losses, registered a lower statistical significance (P<5%) with respect to B_{20} in tomato and lettuce (Tab. 3, contrast 3). B_{20} and B_{10} treatments did not differ significantly each other in chicory and lettuce, while they were still very apart (P<1%) in tomato (Tab. 3, contrast 4).

The further addition of biochar to the soil in the second year of trial (on September 2015, beside September 2014) did not contribute significantly to the environmental N balance in the crop cycle of lettuce. This was observed on both B_{20}^{+} (Tab. 3, contrast 6) and B_{10}^{+} (Tab. 3, contrast 7).

Finally, when a limited N fertilization rate ($N_{1/2}$) was applied to soil, no significant effect was detected in comparing the two opposite biochar addition rates, such as B_0 and B_{20} (Tab. 3, contrast 5).

When the N uptake by the three crops was considered (N_C term of the N balance), no significant differences were detected with respect to biochar and N applications (data not showed) within each experimental crop, although highly significant were the differences among the cultivated species (64, 103 and 25 kg ha^{-1} of N on average in chicory, tomato and lettuce, respectively). This confirms that the double amount of N application should be considered ineffective in terms of plant growth and crop yield, thus proving that (at least in these particular experienced conditions) the entire rate of N applied by the farmer was not in tune with the needs of the crops, thus causing excessive N conditions in the soil and a high risk of N losses.

N leaching is, usually, the prevailing form of N loss in agriculture. Leaching depends, primarily, on NO_3-N availability and its concentration into the circulating soil solution. This concentration is directly related to N supplied as fertilizer. Another relevant factor affecting N leaching pertains to the volumes of drainage water conveying soluble nutrients. In turn, it depends on the total amount of water supplied, both by rainfalls and irrigation [32]. It could be concluded that the greater is the N fertilizer supplied, the lower is the crop uptake rate, the greater is the total water provided to the crop, then it follows the greater is also the consequent risk of N losses. This condition is clearly showed in Figure 2 where N potential losses detected for the three horticultural crops (exemplified by the ΔN_{ENV} values corresponding to the B_0 treatments) are graphically represented as a function of N fertilization and water supplied, respectively. The ΔN_{ENV} amount was progressively greater as the two variables increased their values, from chicory, to lettuce and tomato.

The experimental outcomes allow the following considerations. Performing N balance is a powerful operational procedure in quantifying the right amount of N to be applied by farmer during the cropping season, trying to tune the fertilizing requirements of the cultivated crops. Because of the unavoidable approximations of the method, the need to proceed in estimating some of the balance terms, the intrinsic variability of these terms, and considering the probability that the seasonal course of the crop cycle may be not favorable to plant growth, the chances to overestimate N applications frequently occur.

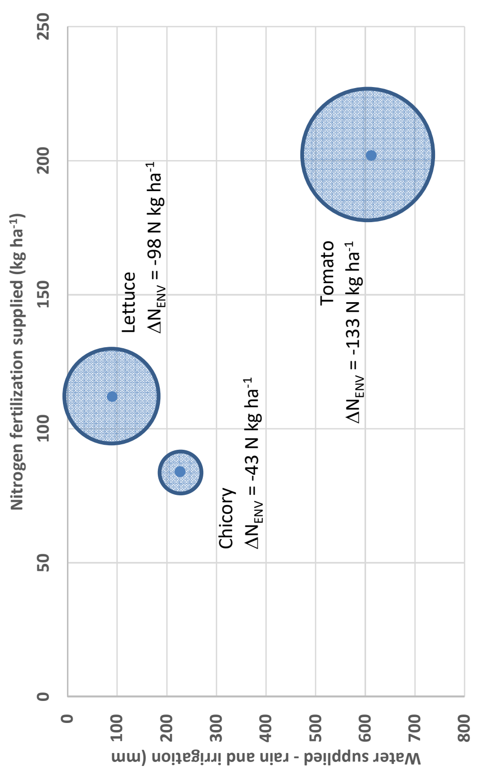

Figure 2. N potential losses for the three considered crops, in terms of ΔN_{ENV} values observed in B_0 (non-amended soil).

These conditions significantly increase the risks of N losses from the soil.

Negative, but close to zero, or slightly positive ΔN_{ENV} values are a clear indication of a well-tuned N fertilizer application, so that the risks of N losses are very limited or under control. Conversely, large negative ΔN_{ENV} values are a clear warning of high environmental risk of N pollution, mostly due to leaching but, possibly, also to atmospheric N emissions.

N supply was the main influencing factor on the N loss risk. This means that farmer's ability in tuning the right amount of N fertilization rate applied to crop (according to its estimable yield and soil conditions) is the main management operation.

Biochar actively contributed in "buffering" possible N losses when a potential N excess was detected. When N applications were controlled or better regulated, then the effect of biochar was drastically reduced and no significant differences were detected in comparing amended and not- amended soils. With particular reference to the retention of N in biochar amended soils and reduction of N losses, several mechanisms have been proposed. These include adsorption of NH_3 or organic-N onto biochar, cations or anion exchange reactions, N immobilization due to the presence of labile C in biochar, inhibited nitrification, incorporation of NO_3^- present in soil solution into biochar pores, increased water retention in the soil and consequent leaching process reduction [12, 33].

4. Conclusions

N fertilization is an essential agronomical input, very useful to gain the crop productive potential, sustaining plant growth and stabilizing yield. In order to increase the effective use of N applied to soil by plants, thus greatly improving the N-use efficiency, fertilizer applications should be properly quantified and timely performed. To save N from losses means not only to save cultivation costs and improve farmer profits, but also reduce (hopefully to cancel completely) the consequent environmental impacts currently jeopardizing both groundwater (through NO_3^- leaching) and atmosphere (mainly through NO_2 emissions).

On this respect, biochar was tested as soil amendment and the focus of the three consecutive crop cultivation trials was to check the effect of biochar on the N balance of the three crop growing cycles. The effect of biochar in mitigating N losses from an intensive cultivated soil was noticeable when N fertilization rate was not properly tuned with plant uptake and larger amount of fertilizer were supplied, thus generating high probability of N losses.

This specific behaviour of biochar in interfering in the soil N balance observed during the experimental period allows to conclude that it acts as a sort of "safety valve", effectively mitigating the risk of N losses and alleviating both their occurrence and entities. Therefore, biochar as soil improver can be considered an effective tool to retain N in the soil.

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