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One-year monitoring of nitrogen forms after the application of various types of biochar on different soils

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ABSTRACT

Biochar is a carbon rich product obtained from pyrolysis of biomass. The use of biochar as soil amendment has been boosted in the last years due to its possible influence on fertility, including its potential ability to lower mineral nitrogen losses, but specially for its potential to reduce greenhouse gases and to increase carbon sequestration in soil. However, the studies on the effects of biochar on nitrogen forms in soil are heterogeneous and contradictory. The present work aims to clarify this point by applying 6 different biochars (with different origin and production process) on 6 different soils (of different properties). The amendment corresponded to an agronomic addition rate of 30 Mg ha^{-1}, together with the addition of urea at a 100 kg N per ha rate. Then those mixtures were incubated for one year at a 60% of the WHC. The samples were analyzed for nitrogen forms (Kjeldahl-N, ammonium-N, nitrate-N, nitrite-N, and microbial-N) at different incubation times (1 week, 1 month, 4 months and 1 year after the addition). The results showed that the effects of different biochars on the soil nitrogen forms were variegated, mainly attributable to soil properties, and to a lesser extent to the particular biochar used. Overall, the Kjeldahl-N (KN) decreased after the incubation time, and only the mixtures with N-rich biochars achieved slightly higher KN compared to controls. Also, biochars tended to induce a decrease in NH_4^+-N, and, especially, in NO_3^--N. The biochars causing highest shifts on N inorganic forms were those produced from agronomic sources (olive and corn wastes) and the one from pine wood materials subjected to high pyrolysis temperature conditions.

1. Introduction

Biochar is a solid, carbon rich product, obtained by thermal treatment of biomass, generally of vegetal origin, at temperatures usually between 300 and 1000 °C (Verheijen et al., 2010). This treatment, known as pyrolysis, is carried out in an atmosphere with low or no oxygen concentration (Lehmann and Joseph, 2009). The major component of biochar is carbon, in addition to oxygen, nitrogen and other elements, in the form of quite stable alkyl and aromatic compounds (Zhang et al., 2009).

The intensification of agriculture has contributed to increased nitrogen fluxes (Galloway et al., 2003). Much of the applied nitrogen is lost to other compartments by different pathways (Davidson et al., 2011), which not only reduces yields but also impacts on the environment and on human health. In this regard, the application of biochar to soil has been associated with different benefits: as a conditioner due to its influence on the soil physical and chemical properties, and its ability to improve the soil fertility (Zhongxin et al., 2017), for the reduction of greenhouse gases (CO_2, CH_4 and N_2O), but mainly because of its ability to sequester carbon (Sohi et al., 2009, Verheijen et al., 2010). The use of biochar has also been claimed to reduce losses of soil nitrogen by volatilization (Cayuela et al., 2013; Zhang et al., 2021) and leaching (Xu et al., 2016), in the last case minimizing the eutrophication of surface water and the presence of nitrates in groundwater.

The potential effects of biochar on soil nitrogen dynamics and on plant development are very relevant when its use as soil amendment is considered (Reverchon et al, 2014). Biochar can affect the mineralization of nitrogen and sorption processes and, therefore, the relative abundance of inorganic forms of nitrogen in the soil (SIN). The results reported in the available literature regarding the effects of biochar on

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soils are heterogeneous and even contradictory. Generally speaking, nitrate leaching and N_2O emissions from soils are reduced, but these effects are not consistent (quantitative or qualitatively) between different types of biochar (Borchard et al., 2019). Soil properties seem to be even more determinant than biochar's in the nitrogen processes (Teutscherova et al., 2018; Hailegnaw et al., 2019). Similarly, Nguyen et al. (2017) in a meta-analysis based on 56 previous studies published between 2010 and 2015 and reporting biochar effect on SIN, concluded that the time of permanence in soil, the application rate, the soil pH, the additional fertilization, and pyrolysis temperature used for biochar production, were the main drivers.

The aim of this work is to clarify the short-term effects of biochar on soil nitrogen dynamics by combining contrasted biochars in terms of feedstock and pyrolysis conditions with soils with variegated properties, under controlled laboratory conditions for 1 year.

2. Materials and methods

2.1. Soils and biochars characterization

Six biochars and six soils were used for the experiments. Five of the biochars were of plant origin and one obtained from sewage sludge. They were produced under varied pyrolysis conditions, as shown in Table 1. In turn, the soils used had contrasting physical and chemical properties and are reported in Table 2.

2.2. Preparation of mixtures, incubation and sampling

The biochars were sieved to 2 mm Ø for mixtures preparation and a subsample grounded for characterization. The soils corresponded to surface horizons (Ap), and were air dried and sieved (2 mm Ø) for their characterization and for mixing. Soil-biochar mixtures were prepared to mimic an application rate equivalent to 30 Mg ha^{-1} (1% w/w). This rate was chosen as representative based on some field studies (Domene et al, 2014). Subsequently, 60 g of the mixture were placed in 100 mL plastic containers, and a water solution of urea equivalent to 100 kg N per ha (1.16%, local maximum fertilizer dose allowed for sensitive areas) was added to provide a suitable soil moisture (60% of the field capacity). All the mixtures were prepared in quadruplicate to allow independent analyses at different sampling times, which corresponded to one week, one month, four months, and one year of incubation.

The control soil samples and the corresponding mixtures were placed in non-hermetically sealed containers, and incubated at 25 °C in the absence of light. Weekly, and along all the incubation process, moisture was restored by weight difference.

Table 1
Biochar feedstocks and pyrolysis conditions, and pollutant burden. PTE: potentially toxic elements, PHC: soluble phenolic compounds (equivalent gallic acid, Folin-Ciocalteau Method), PAHs: polycyclic aromatic hydrocarbons, in GC–MS analysis of toluene Soxhlet extracts.

Code	Origin materials	Time (min)	Temperature °C	N%	Main contaminants
FL	Sludge from urban thermal treatment plant	15	500–550	2.278	PTE (total > 20000 mg kg^{-1}): As, Cd, Zn, Cu, Cr, Ni, Pb
OL	Solid olive wastes from oil production (alperujo)	6	350–400	1.273	PHC: 720 mg kg^{-1}
PG	Sliver from *Pinus maritimus* + *Pinus radiata*	75	600–900	0.210	PAHs: 869 mg kg^{-1}
PL	Sliver from *Pinus maritimus* + *Pinus radiata*	15	500–550	0.177	–
ZL	Corn cob *Zea mays*	120	400–500	0.449	PHC: 304 mg kg^{-1}
QL	Remains of charcoal *Quercus ilex*, *Quercus suber*, *Eucalyptus* sp.	120	400–550	0.454	–

2.3. Samples analysis

The following parameters were determined for each incubation period: Kjeldahl-N (KN), NH_4^+-N, NO_2^--N, NO_3^--N and microbial-N by regular methods (Sparks et al., 1996, Carter & Gregorich, 2007): N by the Kjeldahl method; soluble and exchangeable N-NH_4^+ (0.5 M K_2SO_4 1:5 extracts) by spectrophotometry (Berthelot indophenol blue colorimetric method); water soluble NO_2^--N and NO_3^--N in aqueous (1:10 w/v) extracts by ionic chromatography (Dionex DX300); and microbial-N by the chloroform fumigation extraction method (Carter and Gregorich, 2007). The pH determination was carried out in 1:10 (w/v) water extracts by glass electrode pH meter; soluble salts were analyzed by electrical conductivity (EC) in 1:10 water extracts; and oxidizable organic-C was quantified by the Walkley-Black method.

2.4. Statistical analysis

The statistical analysis consisted of a two-way ANOVA followed by the Tukey test to find significant differences between factors (using as factors incubation time and biochar) at 5% level of probability ($p < 0.05$) (SPSS 21 IBM Corp, Armonk, NY, USA).

3. Results

Due to the volume of data obtained (6 soils, 6 biochars, 4 repetitions of each treatment, 4 incubation periods and 5 parameters analyzed), some results are not shown, but included in the supplementary material together with the significance levels.

While for Kjeldhal-N, ammonium-N and nitrate-N all the results are presented, in the case of nitrite-N and microbial-N only those corresponding to one of the soils are shown as an example to illustrate the comments made in the discussion section.

3.1. Kjeldahl-nitrogen

The results of KN are plotted in Fig. 1. In general, the variation of KN concentrations of the control soils along time showed a different tendency depending on the soil. For some soils it significantly ($p < 0.05$) decreased after one year of incubation, i.e. in AL and RI soils (25 and 17% respectively). The differences in NK for GA soil was not significant, although a clear decreasing tendency was also observed (14%). The rest of soils (higher N content and C/N ratios), did not show any significant effect ($p < 0.05$) on KN concentration along the incubation period. The addition of biochar to the soils did not generally alter their own tendency. So, although the KN content increased with respect to the control (remaining below 0.3%), particularly when adding FL and OL biochars, the subsequent decrease along time was maintained in the soils above mentioned (AL, RI, and also GA in this case). Conversely, three of the biochars (FL, OL, and PG) caused a significant increase ($p < 0.05$) of the KN along the incubation period (4 months and 1 year) in the VI soil (25–33% increase in one year).

3.2. Ammonium-nitrogen

The concentration of NH_4^+-N in soils, with or without biochar, was generally low (<35 mg kg^{-1}), and in three of them it was below 1 mg kg^{-1} (AL, TM, VI soils). The values decreased with time, especially in the period between one week and one month of incubation (Fig. 2), with decreases between 10 and 99%. The highest reductions (95–99%) occurred in the soils with the highest ammonium concentration at the

Table 2
Soils physicochemical properties. O.M.: organic matter. See Section 1.3 for the analytical methods description.

Code	Classification	Clay(%)	pH	$CaCO_3$ (%)	O.M (%)	N (%)	C/N	NH_4^+-N (mg kg^{-1})	NO_3^--N(mg kg^{-1})	NO_2^--N(mg kg^{-1})	Other
AL	Haplic Solonchack	20.8	8.33	21	1.10	0.1011	6.4	9.14	19.2	2.6	saline, calcareous
BA	Andic Cambisol	22.3	6.62	0	4.54	0.2924	9	15.79	10.9	1.7	andic
GA	Haplic Calcisol	24.2	8.24	43	0.58	0.0473	7.2	9.75	24.2	9.0	gypsum, loess
RI	Vertic Luvisol	29.6	7.41	0	1.78	0.1395	7.9	44.60	17.7	6.8	vertic, clayey
TM	Fluvic Cambisol	15.4	8.18	10	1.99	0.1354	8.5	6.22	15.9	10.0	alluvial, loamy
VI	Eutric Arenosol	7.6	7.62	0	1.28	0.0783	9.5	1.12	7.6	2.9	granitic

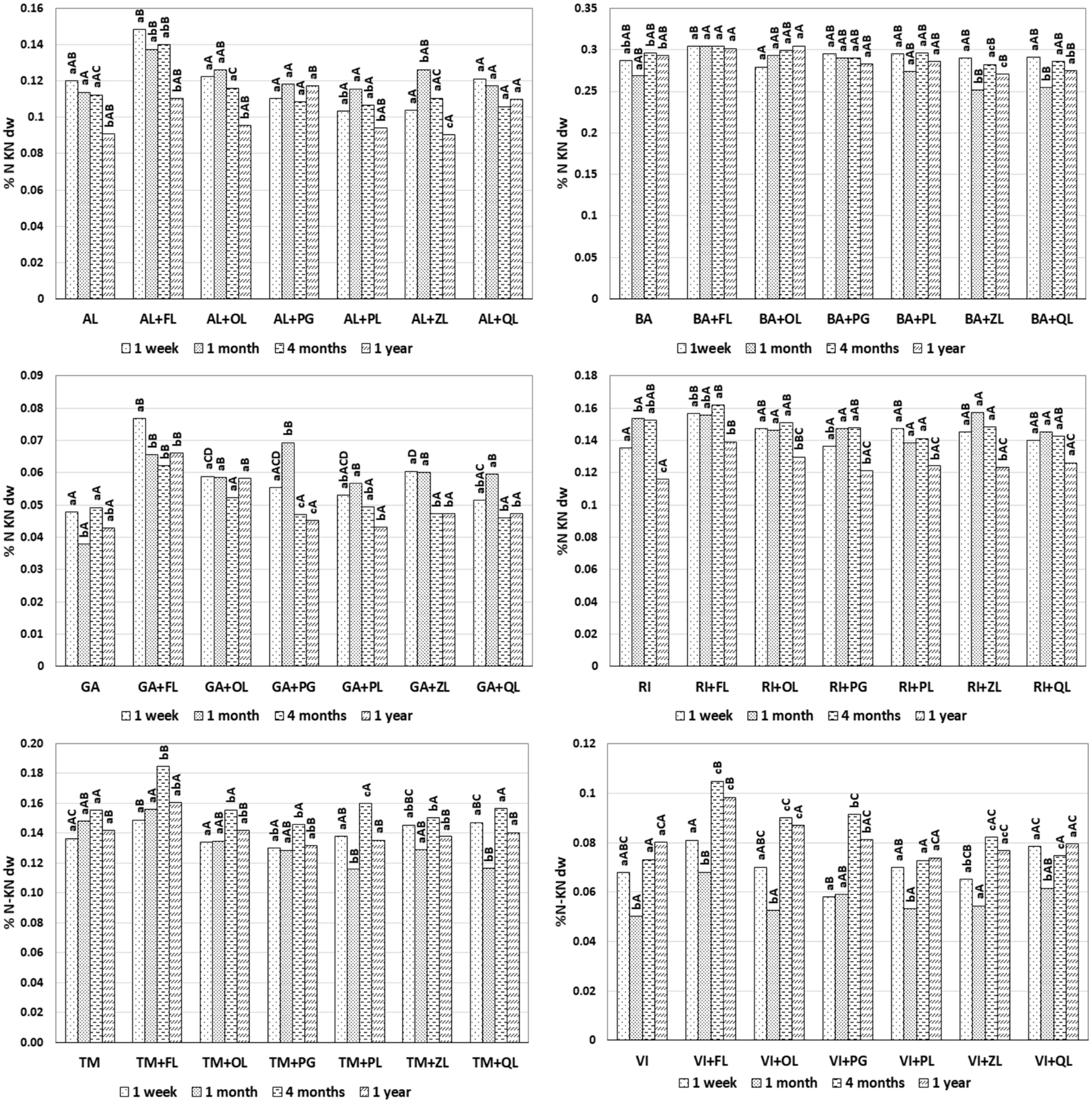

Fig. 1. Kjeldahl-N content (% dw) in the soil-biochar treatments at different incubation times. Lowercase letters indicate significant differences among different sampling times, within each treatment ($p < 0.05$). Capital letters indicate significant differences among different treatments, within each incubation time ($p < 0.05$).

first incubation time. After one month, all ammonium-N values in the control soils leveled off at about 1 mg kg^{-1} or less.

The addition of biochar generally produced a tendency to decrease the ammonium concentration compared to the control at one week of incubation. This decrease was significant for some soils and biochars: The RI soil showed this trend in all the biochars tested, at different intensities, with reductions between around 15 mg kg^{-1} (86%, PG) and 5 mg kg^{-1} (32%, ZL). AL soil was not affected significantly by any of the

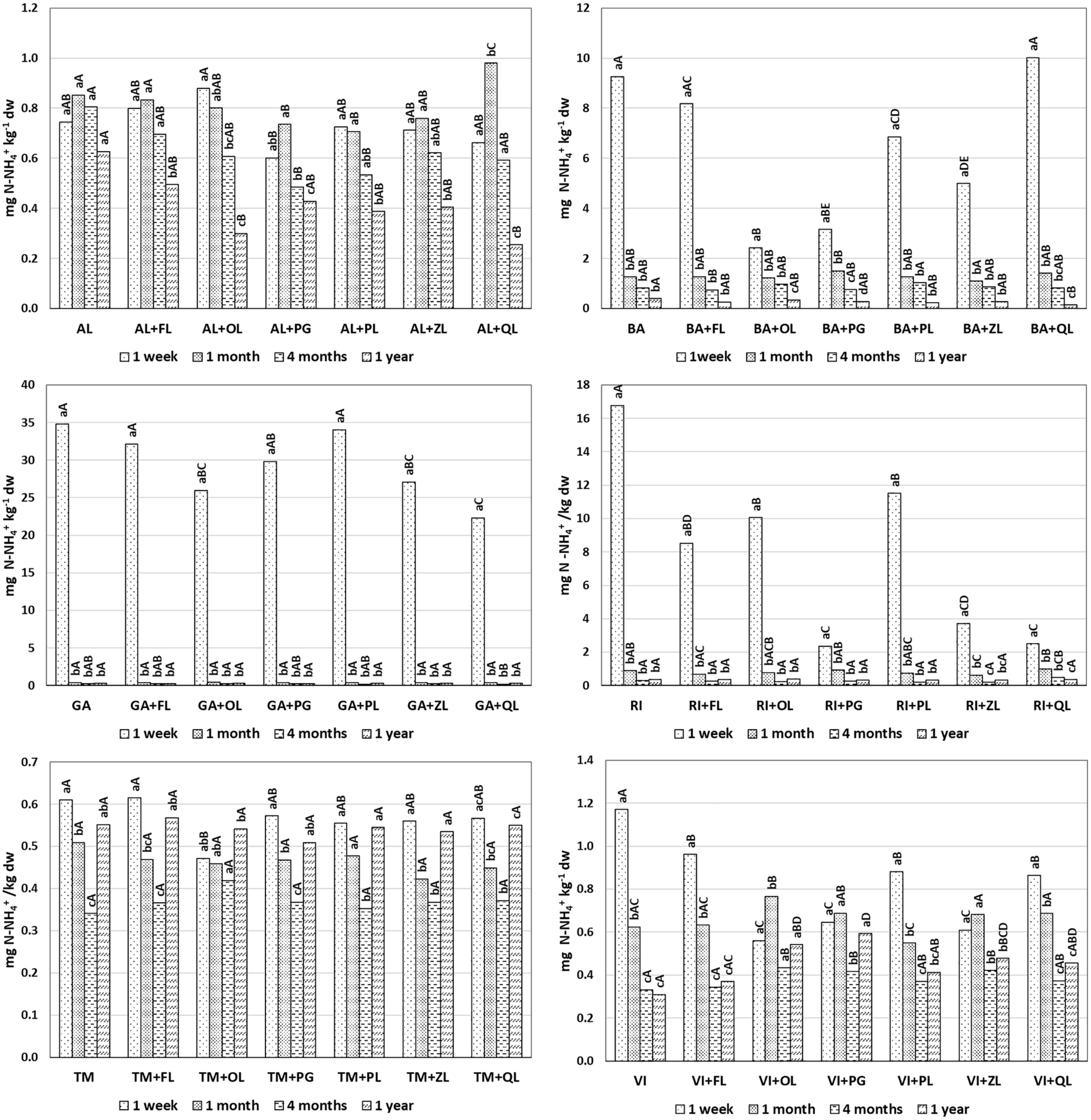

Fig. 2. Ammonium-N content (mg NH_4^+-N kg^{-1}) in the soil-biochar treatments at different incubation times. Lowercase letters indicate significant differences among different incubation time, within each treatment ($p < 0.05$). Capital letters indicate significant differences among different treatments, within each incubation time ($p < 0.05$).

biochars at this first sampling. OL biochar caused this effect on NH_4^+-N in most of the soils, whereas PG and QL were the less influent. Regarding the variation of the concentrations along all the incubation time, NH_4^+-N tended to decrease, and generally speaking, the presence of biochar favored it, with a significant decrease in almost all the incubation times (especially 1 year) and for all biochars, including in the AL soil, the only with no effect on ammonium-N at one week.

3.3. *Nitrite-nitrogen*

As for NO_2^--N, either soils with or without biochar showed concentrations below 20 mg kg^{-1}, but higher than those of NH_4^+-N. As shown in Fig. 3, NO_2^--N peaked after one month of incubation, coupled with the reported rise in ammonium, and subsequently decreased. However, after one year a new increase was observed, which was higher than the one observed at the beginning of the incubation. The increase is remarkably high in the BA soil (250%), high in RI soil (97%) and below 40% in the rest of soil samples. The addition of biochar in almost all cases caused a slight decrease of NO_2^--N compared to the control after one month of incubation (except in the AL and VI soils).

3.4. *Nitrate-nitrogen*

In general, NO_3^--N was the most abundant soluble nitrogenous form in control soils and increased with incubation time. The contribution of biochar did not alter this trend, but it produced a significant decrease (p

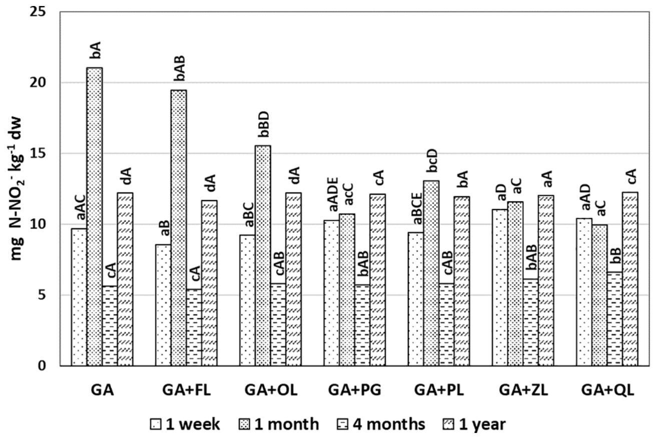

Fig. 3. Nitrite$^-$-N content (mg NO_2^--N kg^{-1}) in GA soil treatments with different biochars at different incubation times. Lowercase letters indicate significant differences among different incubation times, within each treatment ($p < 0.05$). Capital letters indicate significant differences among different treatments, within each incubation time ($p < 0.05$).

< 0.05) in the absolute maximum amounts of nitrate detected compared to the controls (Fig. 4), to a higher extent in the biochar PG followed by ZL, OL and PL. Regarding the relative amounts, at the point of maximum nitrate release (compared to the control soils) biochar addition caused maximum reductions of about 20–30% in BA soil with PG or OL biochars; and in VI soil (biochars PL or ZL). Considering the average reductions for each soil or biochar, the highest rates were observed in soils BA, AL and VI, and for biochars PG and PL.

In some soils (AL and BA), after one year, and independently of the contribution and type of biochar, a decrease in NO_3^--N was observed between the two last incubation times. In these two soils the nitrification rate between one week and 4 months was the highest among all the tested soils, leading to accumulated nitrate concentrations around 200 mg kg^{-1}. This same approximate level of nitrate is not exceeded by any other soil, but for most of them it tends to be reached after one year of incubation, suggesting slower and more sustained nitrification rates.

3.5. *Microbial-nitrogen*

The microbial-N had a variable behavior along time, strongly dependent of the soil concerned. For control BA and RI soils a trend to decreased values along the incubation time was observed (accounting for a total 96 and 42% of decrease after one year, respectively). In these soils, the addition of biochars kept this tendency to decrease but only in BA the decrease was consistent in all samples (Fig. 5). In this soil, for instance, biochar caused a significant decrease in the one-week sampling for PG, PL, ZL, QL biochars (28, 39, 32 and 31% respectively). Biochars caused a more heterogeneous effect in the other soils. Significant increases after 1 month of incubation where recorded for VI soil when some biochars were applied (FL; OL).

4. Discussion

4.1. *Kjeldahl-nitrogen*

The significant decreases in KN over time found in Al and RI soils were probably due to the mineralization of the organic matter throughout the incubation period, favored by their low C/N ratio (Siedt et al., 2021). These soils, together with GA, are the ones with the lowest C/N ratio (<8). However, this trend was not observed in GA soil, probably due to its poor composition (the lowest C and N contents) and thus the lowest microbial biomass expected.

The remainder soils showed no significant differences (1 week to 1 year) in their KN content, probably because they were either fairly balanced soils (nutritionally, e.g. BA, TM soils) or soils with low biological activity, associated with a low N content and high C/N ratio. (i.e. the VI soil). The short time increases in KN when biochar was added were mostly found in biochars with high KN content (FL and OL). Such increases were coupled to the amount of nitrogen added with the biochar (e.g. for FL around 200 mg N kg^{-1}). Some authors (Siedt et al., 2021) have reported that N in biochar tends to be available, whereas C tends to be recalcitrant, thus suggesting a favorable C/N ratio for these materials. However, this is not the unique explanation, and other mechanisms later described might be involved.

The tendency to decrease KN over time is maintained in almost all soils with biochar applications as it did in the control soils. However, the opposite trend can be observed in VI soil, increasing over time, mainly after 4 months of incubation. Such increases in KN when biochar is applied have also been reported by other authors (Prommer et al., 2014; Zhao et al., 2021). The VI soil had a high C/N ratio, and its nitrogen mineral fractions did not undergo any change (ammonium -N remains unchanged and is comparatively low in the total amount of KN), all this suggesting that the increase in the organic N fraction was probably due to N_2 fixation. In this regard, some studies (Harter et al., 2014; Siedt et al., 2021) have reported an increase of N-fixing microbial populations in soils where biochar was applied. High C/N ratios and low mineral nitrogen availability promote this process (Bingham and Cotrufo, 2016), though such effect depends on the type of biochar and soil properties (Zhao et al., 2021). As an example, Zhao et al (2021) attributed the fixation of N to free living N-fixing bacteria, in a plant-free experiment like our study. Other biochar-based explanations such as the provision of habitat, or changes in physicochemical conditions (carbon content, sulphur, molybdenum or other elements) could be also behind this promotion of N_2 fixation. According to Bingham and Cotrufo (2016) when the C/N ratio is high the incorporation of N to the microbial biomass increases. In our study, increases in the microbial biomass N were also recorded in some cases.

4.2. *Ammonium nitrogen*

The low concentrations of NH_4^+-N found in the soils were expectable in the neutral to alkaline soils tested, and probably due to its consumption by the nitrification process.

The decreases caused by biochar addition in our experiment agrees with the results of other authors (Hailegnaw, 2019), that have explained this trend by different processes, such as direct sorption on biochar by different mechanisms (Pal, 2016; Mukherjee et al., 2011; Lopez-Capel et al., 2016) or an enhanced ammonia volatilization (Nelissen et al., 2012; Taghizadeh-Toosi et al., 2012). Cheng et al. (2008) explained the biochar sorption of NH_4^+-N by the increase in the net negative surface charge resulting from the formation of carboxylic and phenolic functional groups with biochar weathering. On the other hand, Schomberg et al. (2012) reported a 5 to 25% increased loss of NH_4^+as gaseous NH_3 when incorporating biochars with pH between 8.7 and 10.3 to soils. This effect could also occur in this case, since the pH range of the biochars used in this work that produce the greatest decrease of NH_4^+-N compared to the control, is between 8.5 and 11.14, though the mixtures hardly reach very high values. In another study with a lysimetric design (Llovet et al., 2021), including one of the mixtures used in our experiment (soil TM with biochar PG), yielded similar results (no significant differences in ammonium concentrations). The lack of effect was attributed to the low level on initial ammonium-N. In the present work this point can be confirmed, as TM soil is only affected by OL biochar, which is the most active biochar in terms of ammonium-N shifts. The soils with the lowest detected effects on ammonium-N when biochar is applied (AL, TM), were those that had initial NH_4^+concentrations below 1 mg kg^{-1}.

As an alternative explanation, we should consider that some of the biochars (OL, PG, ZL) showed high concentrations of free phenolics and PAHs, something that some authors testing biochar from corn pods have partly explained as NH_4^+ reductions through urease activity inhibition

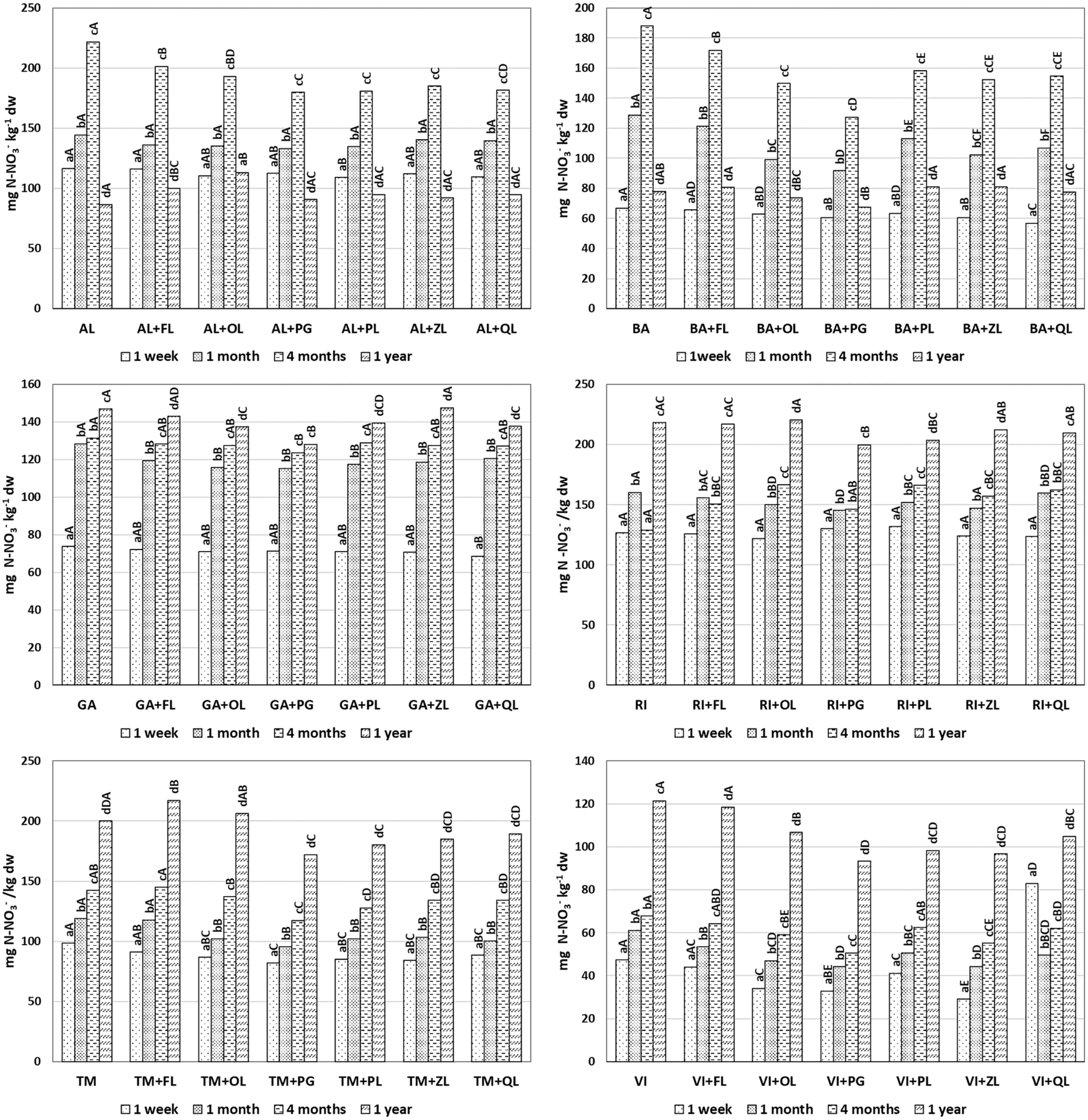

Fig. 4. Nitrate-N content (mg NO_3^--N kg^{-1}) in the soil-biochar treatments at different incubation times. Lowercase letters indicate significant differences among different incubation times, within each treatment ($p < 0.05$). Capital letters indicate significant differences among different treatments, within each incubation time ($p < 0.05$).

(Liu et al., 2018).

4.3. Nitrite-nitrogen

The dynamics of nitrite in soil is highly dependent on its intermediate position in the N mineralization processes. Nitrite-nitrogen production is the first step in the nitrification process (Prommer, 2014; Harter, 2014; Hu et al., 2015), but it is also an intermediate compound in the denitrification pathway (Hu et al., 2015). Nitrites can accumulate in soils, exert some toxicity on plants and microorganisms, and promote greenhouse N gases (Van Cleemput and Samater, 1996) as e.g. N_2O (Venterea et al, 2015). Considering that the nitrification processes in our study do not seem to have been disturbed, with plausible ammonium decreases and nitrate accumulations over the incubation period, nitrite changes are not worth mentioning, in part due to the variability of the results and also considering them within the set of all the nitrogen forms. No significant accumulations were observed over the duration of the experiment that could entail risks as such explained above.

Regarding the effect of biochar addition on the nitrite-N concentrations, a trend to slight decreases compared to the control was observed that might be consistent with the sorption mechanism proposed for NH_4^+. The NH_4^+-N in the soil solution is coupled to the accumulation of nitrite (Venterea et al., 2015), and the biochars tend to produce a decrease in the extractable ammonium as explained in Section 4.2., by sorption processes. Zhang et al. (2021) reported a decrease in nitrite and extractable ammonium in the soils amended with biochar compared to

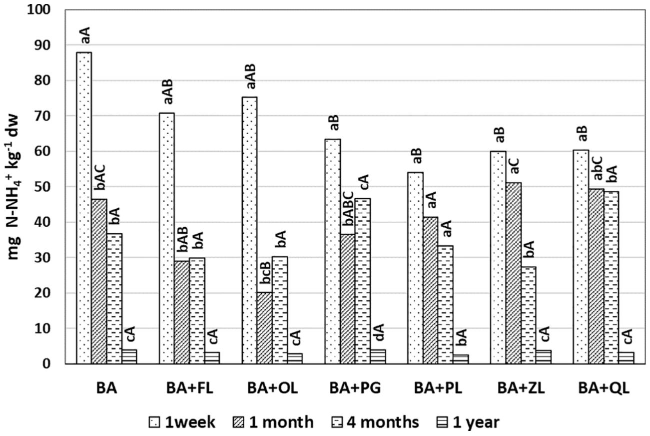

Fig. 5. Microbial-N content (Fumigated/non-Fumigated), expressed in mg NH_4^+-N kg^{-1} in the BA-soil treatments with the different biochars at different incubation times. Lowercase letters indicate significant differences among different incubation times, within each treatment ($p < 0.05$). Capital letters indicate significant differences among different treatments, within each incubation time ($p < 0.05$).

the control soils in a field experiment testing with urea and biochar, parallel to a decrease in N_2O evolution. The authors attribute this effect to the alteration of soil physicochemical properties (i.e., pH, DOC and N availability), and N related microbial activities. However, in our study, the attribution of this decrease to relevant shifts in the nitrification process seems unlikely, as discussed in the nitrate-N section

4.4. Nitrate-nitrogen

In general, a decreasing effect on the maximum extractable nitrate-N was found in the biochar amended samples, compared to their respective control soils. This is one of the expected effects of biochar on soils. Borchard et al. (2019) in a *meta*-analysis reported decreases in the soil nitrate concentrations, mainly after one month, due to biochar amendments. The nitrate concentrations decreased especially in biochars produced at < 500 °C. This could only agree with our results for the OL biochar, but not for the most influent one (PG), which was produced at very high temperatures. We found varied effects in the different soils and biochar combinations, that are difficult to attribute to a single property, and therefore do not coincide with those reported in the aforementioned work. Even so, the general trend to decrease the nitrate concentrations in soils would be coincident with our results, and could be explained by sorption phenomena, as well as other mechanisms (Llovet et al., 2021). Despite the difficulties to do strong statements, we found that the biochar produced by high-temperature pyrolysis (PG) had the greatest effect, being the one containing high concentrations of polycyclic aromatic hydrocarbons (PAH). Others such as ZL, OL had high levels of soluble phenolic compounds (PHC). In both cases these are compounds known to hinder nitrification processes. Wang et al. (2015) in an investigation with comparable biochar-soil rates, proved that the presence of PHC in biochars decreased ammonium oxidizing bacteria (AOB) abundance and, consequently, nitrification decreased with respect to the control soil. Che et al. (2015) indicated that PAH, pH and substrate availability also inhibited AOB in soils. In addition to the previous mechanisms, the effect on urease activities might have a role on the ammonium delivery, as already highlighted.

This decreased nitrate content in AL and BA control soils, totally independent of the presence of biochar, could be related to a phenomenon of microbial immobilization or denitrification, though biochar has been shown to exert some effects leading to decreasing nitrate concentrations (Clough et al., 2013). However, the C / N ratio of these soils would not justify the immobilization of nitrogen by microorganisms and it does not seem very likely that the incubation conditions are conducive to denitrification, unless there were some microsites with anaerobic conditions that facilitate it, or a limitation in the experimental arrangement that could not cope with the medium-term high speed in nitrification of these two samples. For these cases, the conclusions can be taken parallel to the rest of soils regarding nitrate accumulation but considering the time with the maximum nitrate concentrations, which were significantly decreased by biochars addition.

4.5. Microbial-nitrogen

The decreases of microbial-N registered in our experiment for some soils were somehow proportional to the oxidizable carbon content of the soil. The biochar addition had a highly heterogeneous effect, though for some soils a decrease over time was observed (BA and RI soils). This biochar effect could be related to a decreased microbial abundance or nitrogen incorporation to microbial biomass, due to the strong reduction of the labile N initially supplemented as urea after an intense initial nitrification process. According to some authors (O'Neill et al., 2009; Jin, 2010) the usual contribution of biochar is stimulating microbial biomass and activity, although it can also change the structure of the community, while some authors have reported a clear decrease in microbial-N (Zhang et al., 2014). Based on these findings, it seems that the most plausible explanation for the observed trend is a decreased incorporation of nitrogen in the microbial biomass, particularly in soils and biochars with a higher oxidizable carbon content. This finding corroborates the absence of nitrogen immobilization by the microbial biomass, which has been suggested to justify the reduction of NO_3^--N in the AL and BA soils after one year of incubation.

4.6. Final considerations

The results of this study show the difficulty of attributing effects on the nitrogen forms of the soil univocally to the biochar. Not only due to the diversity of biochars and their composition and properties, but also due to the diversity of properties and situations present in the recipient soils. Although general trends can be seen in some parameters (such as nitrates or ammonium), most situations depend on the precise combination of soil and biochar, in addition to the contact time of both. This variability, or inconsistency of results, pointed out by various authors (Teutscherova et al., 2018; Hailegnaw et al., 2019) especially for some nitrogen dynamics, entails the need to analyze each situation case-by-case (Clough et al., 2013) when planning the use of biochar in soil for N cycle regulation, but also to warn on the need for studies modeling N forms response to biochar and soil properties to identify quantitatively and qualitatively which parameters must be taken into account.

5. Conclusions

The effects on soil nitrogen forms derived from the application of different biochars to different soils, and evaluated during one year, were highly variable. Most of the effects observed were mainly attributable to the characteristics of the soils studied and only eventually to the biochar. The Kjeldahl-N of control soils decreased after one year of incubation in the soils with lower Kjeldahl-N content and C / N ratio, probably because of the mineralization of the organic matter. The addition of N rich biochars was linked to higher Kjeldahl nitrogen contents than control plots along the incubation. The concentration of NH_4^+-N in soils, irrespective of the addition or not of biochar, was generally low and decreased with time, probably as a consequence of nitrification, and especially in the period between one week and one month of incubation. The addition of biochar decreased NH_4^+-N concentrations compared to controls, and plausibly resulting from ammonium sorption or volatilization as ammonia. In general, NO_3^--N was the most abundant soluble nitrogenous form in control soils and increased with incubation time. After one month and four months of incubation, biochar caused a notable decrease in NO_3^--N compared to controls probably due to sorption phenomena and / or the presence of contaminants inhibiting the nitrification process. The microbial nitrogen decreased with the incubation time, more sharply in soils with higher organic matter content and, probably, higher biological activity.

The biochars with the greatest influence on the evolution of inorganic forms of soil nitrogen were OL (from olive wastes), PG (pine wood, high temperature), and ZL (Corn pods). These biochars are characterized by their high content of PAH and PHC, which can be associated with the nature of biochar (OL and ZL) and to the pyrolysis process (PG).

In this study, using a wide diversity of combinations of soils and biochars, we demonstrated the difficulties to establish clear and universal biochar effects on soil nitrogen forms. On the contrary, our results highlight the need for case-by-case studies for every biochar to be used in a particular soil. Furthermore, the construction of models relating soil and biochar properties from the available published studies, including the ones of our study, constitute the way to find such universal trends and enable theoretical predictions of N-cycle effects of biochar addition.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115178.

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