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Nitrogen mineralization and microbial biomass carbon and nitrogen in response to co-application of biochar and paper mill biosolids

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ABSTRACT

Adding biochar to paper mill biosolids (PB) amendments may affect PB mineralization rate and nitrogen (N) availability. The objective of this 224-day incubation study was to evaluate the effect of amending two PB types varying in carbon (C)/N ratio (PB1, C/N = 24; and PB2, C/N = 13) with three rates (0%, 2%, and 5%) of pine (*Pinus strobus* L.) biochar produced at 700 °C on the dynamics of total C, total N, mineral N, N mineralization rate, and microbial biomass C (MBC) and N (MBN) in two agricultural soils (Kamouraska clay and St-Antoine sandy-loam). Two reference treatments were also included, namely, mineral fertilization and unamended soil. Total soil C concentration remained stable over the incubation period, whereas a decrease in total soil N was observed in both soils. In comparison with the unamended soil, the application of PB significantly increased total N, NH_4-N, NO_3-N, net mineralized N, applied N mineralization rate, and MBC in both soils. In comparison with the application of PB alone, biochar addition increased total C and MBC but decreased NH_4-N, NO_3-N, net N mineralization, and applied N mineralization rate in both soils. The co-application of biochar and PB1 resulted in the sequestration of mineral N released, which was more pronounced in the Kamouraska clay soil. The co-application of biochar and PB2 resulted in moderate release of mineral N. This study showed that the co-application of biochar and PB can benefit agricultural soils by improving NO_3-N retention in agroecosystems while increasing organic matter and promoting microbial biomass.

1. Introduction

Organic amendments can provide essential nutrients to crop plants and improve soil physical quality (Scotti et al., 2013). The application of organic materials to agricultural soils is an environmentally beneficial waste disposal practice (Bellamy et al., 1995; Zibilske et al., 2000). In Quebec, it is estimated that about 1.5 million tonnes of fertilizing residual materials was applied to agricultural soils in 2015, with paper mill biosolids (PB) accounting for 23.4% of this amount (MDDELCC, 2016). The application of PB to agricultural soils has been practiced for decades in Canada (Bellamy et al., 1995; Simard, 2001).

When applied to agricultural soils, PB supply nutrients, restore soil organic matter levels, improve soil fertility, structure, and water-holding capacity (Bipfubusa et al., 2005; Zibilske et al., 2000), and promote microbial biomass and activity, thus increasing crop yields (Camberato et al., 2006; N'Dayegamiye, 2006). However, the effects of PB application on soil water-holding capacity and organic matter level do not last long, because the organic matter applied is usually mineralized rapidly and, therefore, only a small portion of the applied PB is stabilized in soil in the long term (Bipfubusa et al., 2008; N'Dayegamiye et al., 2004a). Regular PB inputs are recommended to increase or maintain soil organic matter levels (Gagnon and Ziadi, 2012; N'Dayegamiye et al., 2004a). Unfortunately, this practice may also contribute to the leaching of nutrients, especially NO_3-N, when PB are applied annually at high rates (Gagnon et al., 2010). The co-application of PB with stable carbon (C) such as that contained in biochar could be a way to increase soil organic matter levels while limiting nutrient leaching (Xu et al., 2016).

Abbreviations: C, carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N, nitrogen; PB, paper mill biosolids; PB1, paper mill biosolids with C/N = 24; PB2, paper mill biosolids with C/N = 13

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Biochar is produced from the pyrolysis of organic feedstocks such as wood, crop residues, and manure (Lehmann and Joseph, 2009; Zimmerman et al., 2011). During this thermal degradation, the organic portion of the feedstocks is converted to solid (biochar), liquid, and gaseous fractions (Lehmann and Joseph, 2009). The growing interest in biochar as an amendment for agricultural soils lies in its potential to mitigate climate change through C sequestration (Wang et al., 2015; Zimmerman et al., 2011) and the reduction of CH_4 and N_2O emissions (Hangs et al., 2016; Lévesque et al., 2018).

It has been reported that co-applying biochar with manure (Ippolito et al., 2016) or compost (Hagemann et al., 2017) was found to produce better effects on soil properties over the application of biochar, manure, or compost alone. To our knowledge, the effect of co-applying biochar and PB on soil properties has not yet been evaluated, even though PB are already commonly applied to agricultural soils. The variable composition and C/N ratio of PB control their mineralization rate and thus the release of the nutrients that they contain, especially N, and their contribution to soil organic matter (Joseph et al., 2017; N'Dayegamiye et al., 2004b; Ziadi et al., 2013). Biochar application to soils can impact soil nitrogen (N) dynamics and improve NO_3-N retention in agroecosystems (Hagemann et al., 2017; Wang et al., 2015). Biochar application can also affect the structure and activity of soil microbial communities and, thus, the cycling of nutrients, especially C and N (Lehmann et al., 2011; Wang et al., 2015). There is a need to determine whether biochar addition to PB-amended soils could impact the dynamics of soil N and reduce nitrate leaching.

The objective of this study was to assess the effects of the co-application of PB and biochar on soil N dynamics and microbial biomass. Our hypotheses were that co-applied biochar would modify the impact of PB on total soil C and N, soil NH_4-N and NO_3-N, net N mineralization, applied N mineralization rate, and soil microbial biomass C (MBC) and N (MBN), and that the influence of biochar co-application would depend on biochar rate, PB type, and incubation time.

2. Materials and methods

2.1. Soils, paper mill biosolids and biochar characteristics

Composite soil samples were collected in spring 2011 from the upper layers (0–15 cm) from two fields in the province of Quebec, Canada. A Kamouraska clay (fine, mixed, frigid, Typic Humaquept) (Soil Survey Staff, 2010) was collected from an agricultural field in Lévis (46° 47′ N, 71° 08′ W), near Quebec City, and a St-Antoine sandy-loam (loamy, mixed, frigid, Dystrochrept) (Soil Survey Staff, 2010) was collected from an agricultural field in St-Nicolas (46° 41′ N, 71° 28′ W), also near Quebec City. The Kamouraska clay had been under corn (*Zea mays* L.) since 2008, while the St-Antoine sandy-loam had been under corn in 2006 and 2008, soybean (*Glycine* max L.) in 2007, and pasture (45% alfalfa [*Medicago sativa*] and 55% timothy [*Phleum pratense* L.]) in 2009 and 2010. Field moist soil samples were kept frozen (−20 °C) for about 1 year. They were then thawed, passed through a 6-mm sieve, air-dried and kept at room temperature (25–30 °C) (St. Luce et al., 2016). At the beginning of the experiment (April 2017), the soil was passed through a 2-mm sieve, and then physicochemically analyzed (Table 1).

The first PB (PB1) had been kept frozen since 2001. Those PB were obtained from Kruger Inc., Trois-Rivières, Quebec, Canada, and came from a thermomechanical pulping process. The second PB (PB2) had been obtained in 2008 from Kruger Wayagamack Inc., Trois-Rivières, Quebec, Canada, and kept frozen until use. Those PB came from a kraft pulping process involving acid treatment and bleaching (Gagnon and Ziadi, 2012). The two PB were physicochemically characterized (Table 2). The biochar used (Table 2) was produced from pine chips at 700 °C, was purchased from Biochar Engineering (Golden, CO, USA), and was described by Lévesque et al. (2018).

2.2. Experimental design and treatments

The effect of three rates (0%, 2%, and 5% w/w) of biochar co-applied with each of two PB (2.5% w/w) on the Kamouraska clay and St-Antoine sandy-loam soil was investigated during a 224-d incubation study. The experimental design was a randomized complete block with eight treatments: unamended soil, mineral-fertilized reference treatment, PB1 without biochar, PB1 + 2.5% biochar, PB1 + 5% biochar, BP2 without biochar, PB2 + 2.5% biochar and PB2 + 5% biochar. There were three replications of each treatment for each sampling date (7, 14, 21, 28, 56, 112, and 224 days) so that destructive sampling could be carried out, for a total of 336 jars.

Biochar, fresh PB and mineral fertilizer solution were thoroughly mixed by hand with the soils. The mineral fertilizer treatment consisted of 120 kg N ha^{-1} using NH_4NO_3 and 30 kg P ha^{-1} and 37 kg K ha^{-1} using KH_2PO_4, which are the rates recommended for the fertilization of corn produced in Quebec, Canada (CRAAQ, 2010). Each experimental unit consisted of 100 g (dry weight equivalent) of treated soil in a 500-mL Mason jar moistened to 60% water-filled pore space with deionized water for optimal microbial activity (Linn and Doran, 1984). The lids of the jars were inverted to allow aeration while limiting the loss of moisture. The jars were incubated in the dark at 25 °C. Moisture was adjusted twice a week by weighing the jars and adding deionized water as necessary. At the prescribed incubation time, a series of 48 jars (2 soils × 8 amendments × 3 repetitions) was taken, and the treated soils were analyzed. Soils not immediately analyzed were kept refrigerated at 4 °C for 24 h.

2.3. Soil analyses

The treated soils were analyzed for total C and N, NO_3-N, NH_4-N, MBC, and MBN. Total C and N were determined by dry combustion (LECO TruSpec CN; Leco Inc., St. Joseph, MI, USA) of soil samples ground to 200 μm. Soil NO_3-N and NH_4-N were extracted by shaking 2 g of soil in 20 mL of 2 *M* KCl for 30 min (Maynard et al., 2008), and the extract was analyzed with an automated continuous-flow injection colorimeter (QuickChem 8000 FIA+; Lachat Instruments, Loveland, CO, USA). Soil MBC and MBN were determined according to the method of Voroney et al. (2008). Briefly, one of two sets of fresh soil samples (10 g) was fumigated for 24 h with chloroform in the dark. The fumigated and non-fumigated samples were extracted with 20 mL of 0.25 *M* K_2SO_4 (extraction ratio between 1:5 and 1:2). The extracts were analyzed using a total organic C analyzer (TOC-V Series; Shimadzu

Table 1
Chemical characteristics of the soils used in the incubation study.

	pH	Sand[a]	Silt[a]	Clay[a]	Total C	Total N	C/N	NO_3-N	NH_4-N	P	K	Ca	Mg	Al	Fe	Mn	Cu	Zn	Cd
		g kg^{-1}						mg kg^{-1}											
Kamouraska clay	5.32	302	292	406	30.2	2.46	11.89	2.0	17.6	36.3	108	2569	302	1284	206	116	4.5	10.7	0.181
St-Antoine sandy-loam	5.89	683	164	152	16.3	1.27	13.2	2.2	53.9	32.7	129	1068	164	1107	391	14	2.1	1.4	0.035

[a] Data from St. Luce et al. (2016).

Table 2
Physicochemical characteristics of the paper mill biosolids and biochar used in the incubation study.

	CEC	pH	Total porosity	Moisture	Total											C/N	NO_3-N	NH_4-N	PO_4-P
					N	C	P	K	Ca	Mg	Fe	Al	Mn	Cu	Zn				
	cmol kg^{-1}		cm^3 cm^{-3}	g kg^{-1}	mg kg^{-1}														
PB1	187.63	7.80	ND[a]	707	13.1	315	4.3	2500	8000	700	ND	8715	1723	5	32	24.1	0.5	1108	72
PB2	162.7	4.51	ND	693	36.4	485	7.4	1000	2200	500	855	ND	148	16	104	13.3	0.4	154	1051
Biochar[b]	96.2	7.4	0.90	68	12.4	761	0.04	2500	6000	1400	2309	1205	361	< 5	38	61.36	1.53	1.04	ND

[a] ND, not determined.
[b] Data from Lévesque et al. (2018).

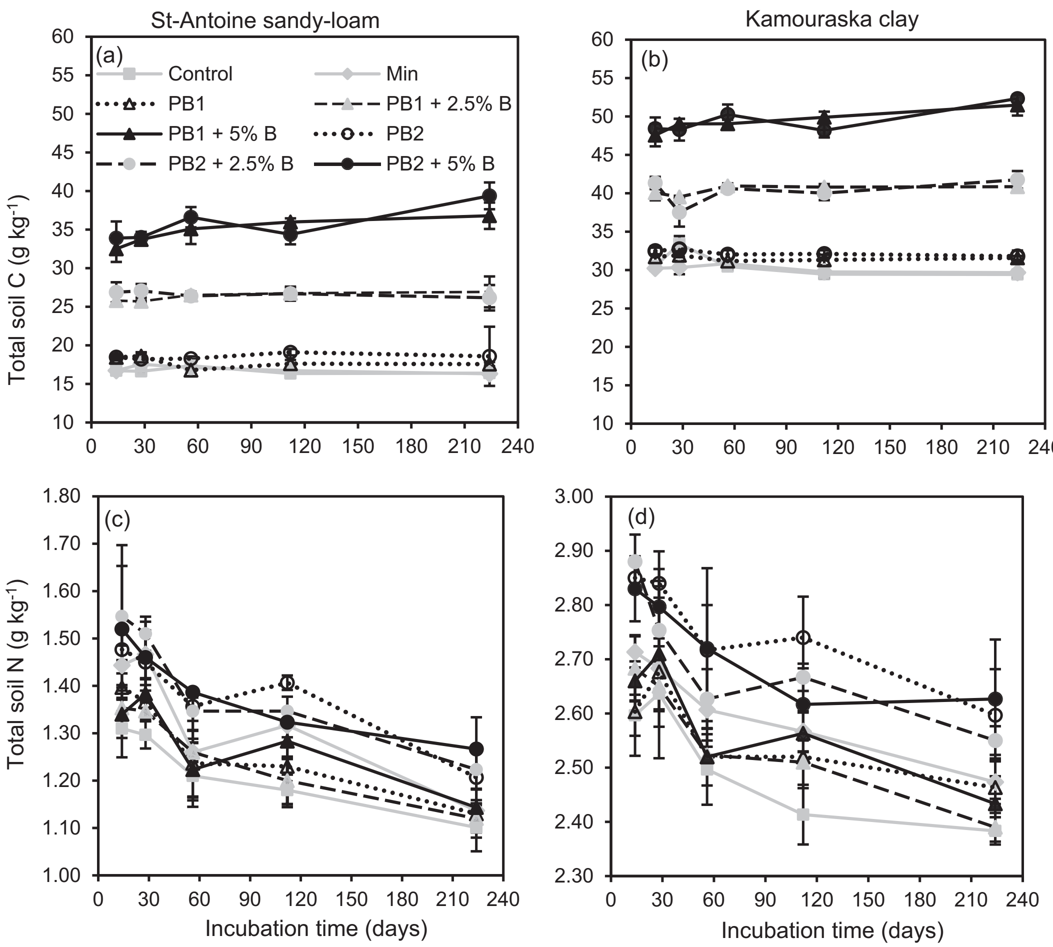

Fig. 1. Variation in total soil C (a, b) and N (c, d) in St-Antoine sandy-loam and Kamouraska clay soils during a 224-day incubation. Measurement was done on days 14, 28, 56, 112 and 224. Bars represent standard errors of the mean (n = 3). Control = unamended soil, Min = mineral fertilization, PB1 + 2.5% B = PB1 + 2.5% biochar, PB1 + 5% B = PB1 + 5% biochar, PB2 + 2.5% B = PB2 + 2.5% biochar and PB2 + 5% B = PB2 + 5% biochar.

Corporation, Kyoto, Japan) with a total N measuring unit (TNM-1; Shimadzu Corporation). The MBC and MBN concentrations were calculated as the difference in total C and N contents in the fumigated and non-fumigated extracts, corrected by an extraction efficiency factor (K_{EP}) of 0.45 for MBC (Wu et al., 1990) and 0.54 for MBN (Brookes et al., 1985).

2.4. Net N mineralization and applied N mineralization rate determination

Net mineralized N was calculated as the difference between the soil mineral N concentration after a sampling period and the initial soil mineral N concentration. Applied N mineralization rate was calculated by subtracting the mineral N concentration of the unamended control soil from that of the amended soils, and the difference was expressed as the proportion of total N applied (Gale et al., 2006). Thus,

$$\text{Applied N mineralization rate } (\%) = (N_{treatment} - N_{control}) * 100/N_{applied}$$

where $N_{treatment}$ is the concentration of mineral N in the amended soil sample on a sampling date; $N_{control}$ is the concentration of mineral N in the unamended control samples on the corresponding sampling date; and $N_{applied}$ is the total amount of N applied through PB application.

2.5. Statistical analysis

All statistical analyses were performed using the Proc Mixed procedure in SAS 9.4 software (SAS Institute Inc., 2015). All data were checked for normality with the Shapiro–Wilk test and transformed when necessary. The data was analyzed according to a randomized

Table 3
Significance of the effects of incubation time and amendment on total C and N, mineral N, applied N mineralization rate and microbial biomass C and N in St-Antoine sandy-loam and Kamouraska clay soils according to ANOVA (n = 3).

	St-Antoine sandy-loam								Kamouraska clay							
	TC[a]	TN[b]	NH_4-N	NO_3-N	Net N mineralized	Applied N mineralization rate	MBC[c]	MBN[d]	TC	TN	NH_4-N	NO_3-N	Net N mineralized	Applied N mineralization rate	MBC	MBN
Source of variation																
Amendments (A)	***	***	***	***	***	***	***	0.080	***	***	***	***	***	***	***	0.625
Time (T)	0.454	***	***	***	***	***	***	***	0.056	***	***	***	***	***	***	***
A × T	**	0.496	***	***	***	***	0.407	0.108	***	0.656	***	***	***	***	*	0.937

* Significant at the 0.05 probability level, respectively; the numbers indicate non-significant P values.
** Significant at the 0.01 probability level, respectively; the numbers indicate non-significant P values.
*** Significant at the 0.001 probability level, respectively; the numbers indicate non-significant P values.
[a] TC, total carbon.
[b] TN, total nitrogen.
[c] MBC, microbial biomass carbon.
[d] MBN, microbial biomass nitrogen.

complete block design. A two-way analysis of variance (ANOVA) was performed to test the effect of incubation time, amendment, and their interaction on total soil C and N, soil N mineral dynamics, and microbial biomass (C and N). Differences were considered statistically significant at $P < 0.05$, according to Tukey's test.

3. Results

3.1. Total soil C and N

The co-application of biochar and PB increased total soil C concentration in both soils (Fig. 1a, b; Table S1) to the extent of biochar application rate. In contrast, the effect of the co-application of biochar and PB on total soil N concentration did not differ from that of the application of PB alone (Fig. 1c, d; Table S1). The application of PB did not affect total soil C concentration in comparison with the unamended control, in both soils (Fig. 1c, d; Table S1). In contrast, the application of PB increased total soil N concentration (Fig. 1c, d; Table S1) to the extent of their N content (Table 2).

Within each amendment and in both soils, total soil C concentration remained stable over the incubation period (Fig. 1a, b; Table S1), as shown by the insignificance of the incubation time effect (Table 3). Total soil N declined similarly with incubation time in both soils (Fig. 1c, d; Table S1), as shown by the insignificance of the interaction between amendment and incubation time (Table 3).

3.2. Soil NH_4-N, NO_3-N, net N mineralization, and applied N mineralization

The interaction between incubation time and amendment significantly influenced soil NH_4-N, NO_3-N, and net mineralized N concentrations and applied N mineralization rate (Table 3). This significant influence explains the variable levels of these parameters during the incubation (Figs. 2, 3; Tables S2, S3). During the incubation period, soil NH_4-N concentration decreased in the St-Antoine sandy-loam soil but increased until day 28 and then decreased thereafter in the Kamouraska clay soil (Fig. 2a, b; Table S2). Conversely, soil NO_3-N concentration (Fig. 2c, d; Table S2) and net mineralized N concentration (Fig. 3a, b; Table S3) increased over the incubation period in both soils. Moreover, a sharp increase in NO_3-N and net mineralized N concentrations (Figs. 2, 3; Tables S2, S3) began to occur from day 28 in the Kamouraska clay soil. The co-application of biochar and PB decreased soil NH_4-N, NO_3-N, and net mineralized N concentrations and applied N mineralization rate (Figs. 2, 3; Tables S2, S3) in comparison with PB-only application in both soils, and the effect was greater in the amendments with the 5% biochar rate. The application of PB alone increased soil NH_4-N, NO_3-N, and net mineralized N concentrations above those of the unamended control in both soils. The application of PB2, with its lower C/N ratio and higher N content (Table 2), resulted in higher NH_4-N, NO_3-N, and net mineralized N concentrations and applied N mineralization rate (Figs. 2, 3; Tables S2, S3) in comparison with PB1 in both soils. The co-application of biochar and PB1 resulted in a negative applied N mineralization rate, which was less pronounced in the St-Antoine sandy-loam soil and which tended to decrease from day 56 in that soil but kept increasing until the end of the incubation in the Kamouraska clay soil. Amendment with mineral fertilization had the highest NO_3-N until day 56 in the St-Antoine sandy-loam soil and until day 28 in the Kamouraska clay soil.

3.3. Soil microbial biomass C and N

Soil MBC increased similarly over time among amendments with incubation time in the St-Antoine sandy-loam soil (Fig. 4a; Table S4), as shown by the insignificance of the interaction between amendment and incubation time (Table 3). In contrast, the interaction between amendment and incubation time (Table 3) was significant in the

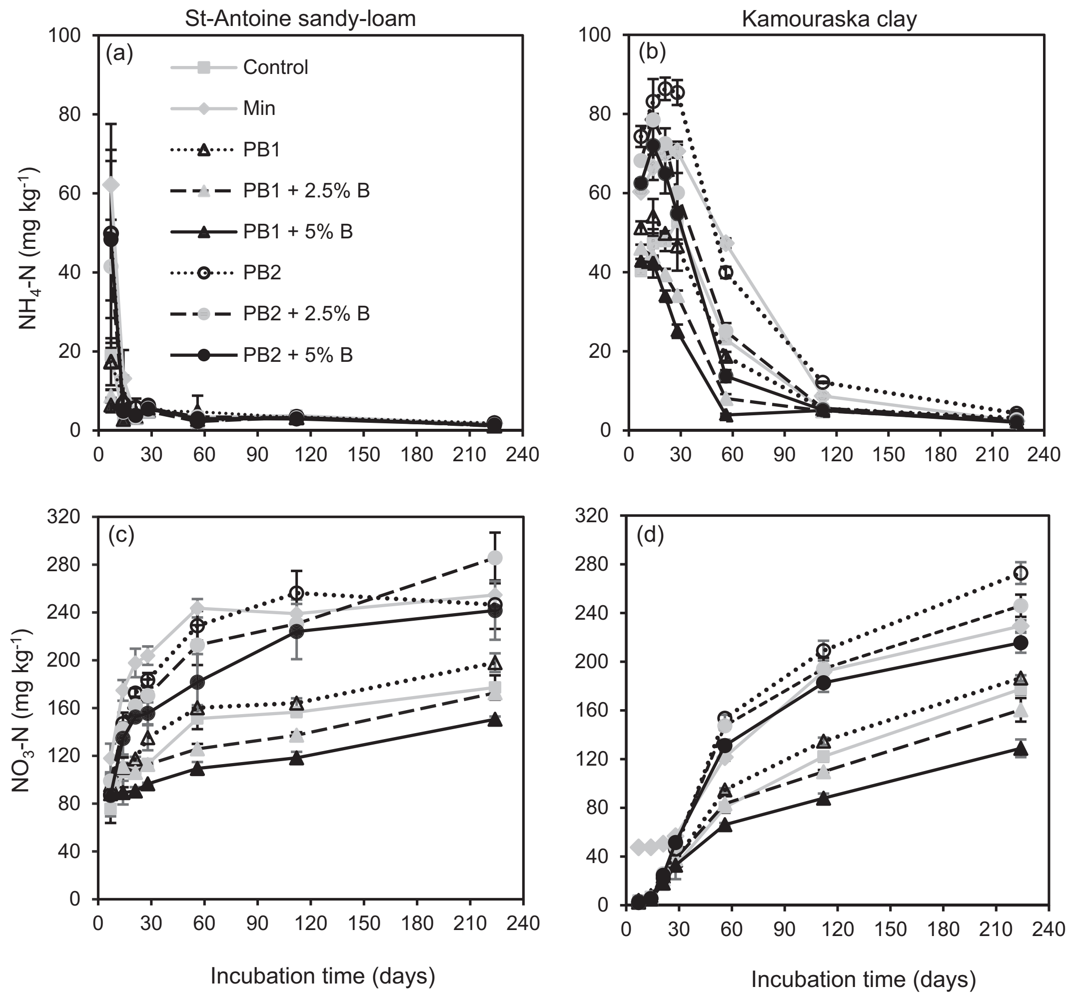

Fig. 2. Variation in NH_4-N (a, b) and NO_3-N (c, d) levels in St-Antoine sandy-loam and Kamouraska clay soils during a 224-day incubation. Measurement was done on days 7, 14, 21, 28, 56, 112 and 224. Bars represent standard errors of the mean (n = 3). Control = unamended soil, Min = mineral fertilization, PB1 + 2.5% B = PB1 + 2.5% biochar, PB1 + 5% B = PB1 + 5% biochar, PB2 + 2.5% B = PB2 + 2.5% biochar and PB2 + 5% B = PB2 + 5% biochar.

Kamouraska clay soil, as the MBC gap between the amendments was greater on day 224 in this soil (Fig. 4b; Table S4). Soil MBC was greater in the amended soils than in the unamended control or in the soils receiving mineral fertilization (Fig. 4a, b; Table S4). In both soils, the co-application of biochar and PB increased soil MBC in comparison with the application of PB alone (Fig. 4a, b; Table S4).

Only incubation time significantly influenced soil MBN in both soils (Table 3). Soil MBN decreased from day 28 to day 112 and increased thereafter until the end of the incubation (Fig. 4c, d; Table S4).

4. Discussion

4.1. Effect on total soil C and N

As expected, the co-application of biochar and PB significantly increased total soil C concentration in comparison with the application of PB alone, probably because biochar is made up mostly of C (Table 2). Our results are consistent with those of Li et al. (2017), who reported an increase in soil C concentration as the rate of apple (*Malus pumila* Mill.) branch biochar addition was increased, regardless of the amount of N applied. Similarly, Lentz and Ippolito (2012) and Plaza et al. (2016) found an increased total soil C concentration when biochar was co-applied with manure and compost, respectively. In contrast, the co-application of biochar and PB did not increase total soil N in comparison with the application of PB alone, probably because of the low N content (Table 2) of the biochar.

The greater increase in total soil N with PB2 was due to its higher total N content (Table 2) in comparison with PB1. Gagnon and Ziadi (2012) made similar observations when applying different types of PB with different total N contents. In their 9-year field study, those authors found significant increases in total soil N concentration when PB with high total N contents were applied in comparison with PB with low N contents. In most cases, soil is N-deficient, and the main source of mineral N is soil organic matter, via N mineralization (Masunga et al., 2016). Thus, in our study, N mineralization seemed to be the main cause of the decrease in total soil N since the beginning of the incubation and within all amendments. On the other hand, total soil C concentration did not decrease in any of the treatments during the incubation. This situation suggests that C kinetics differed from N kinetics and supports the notion that C and N are not necessarily maintained by the same controls (Paul et al., 2011). In this study, soil MBC concentration increased over time suggesting that microbes were decomposing soil organic matter, but this change was not detected in the total soil C pool. It is well known that changes in total soil C are mostly undetectable in the short term, with changes being more readily observed in the labile C pools.

4.2. Effect on N mineralization

Our results confirmed our prediction that the application of PB

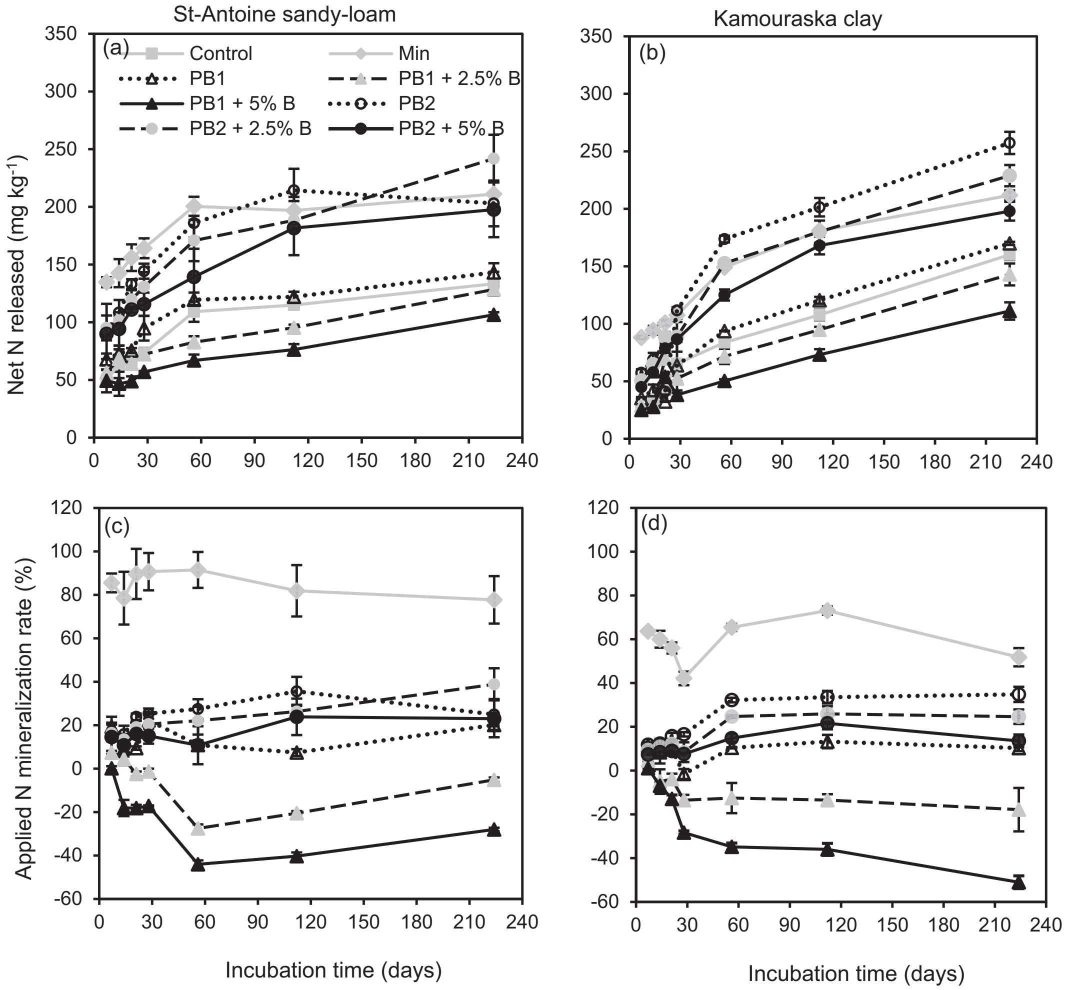

Fig. 3. Variation in net N released (a, b) and N mineralization rate (c, d) in St-Antoine sandy-loam and Kamouraska clay soils during a 224-day incubation. Measurement was done on days 7, 14, 21, 28, 56, 112 and 224. Bars represent standard errors of the mean (n = 3). Control = unamended soil, Min = mineral fertilization, PB1 + 2.5% B = PB1 + 2.5% biochar, PB1 + 5% B = PB1 + 5% biochar, PB2 + 2.5% B = PB2 + 2.5% biochar and PB2 + 5% B = PB2 + 5% biochar.

alone and the co-application of biochar with PB would impact N mineralization differently. Throughout the incubation period, the co-application of biochar and PB significantly decreased soil NH_4-N, NO_3-N, net mineralized N concentration, and applied N mineralization rate in comparison with the application of PB alone. The biochar used in this study was generated by the pyrolysis of pine chips at 700 °C (Lévesque et al., 2018). It is known that biochar derived from high-temperature pyrolysis is characterized by a large surface area, high cation-exchange capacity, and high total porosity (Xu et al., 2012; Yue et al., 2016), which may increase the biochar's adsorption capacity (Xu et al., 2016). The stronger adsorption of free NH_4^+ on biochar particles (Xu et al., 2016) may have caused the decrease in NH_4-N concentration in the biochar-treated soils. This suggests that biochar application may have minimized the nitrification of NH_4-N released from the PB into NO_3-N. The adsorption of NH_4-N released from the PB seems to be the main cause of decreased soil NO_3-N availability and thus decreased net mineralized N in the soils amended with biochar and PB in comparison with those amended with PB alone. Hagemann et al. (2017) showed that mixed woody waste biochar produced at 600 to 700 °C was the only component in biochar-amended composted manure that caused the capture of nitrate and enabled its slow release. The application of biochar to soil can induce N immobilization by providing labile C (Wang et al., 2015; Zimmerman et al., 2011). Lévesque (2017) found no significant increase in dissolved organic C when the same biochar used in our study was applied at a rate of 5% with or without 3.8% compost. This suggests that this biochar alone could not provide enough C to significantly stimulate N immobilization. In comparison with the unamended control, the co-application of biochar and PB1 drastically reduced net mineralized N concentration in both soils. With a C/N ratio of 24, PB1 is poorly mineralizable (Joseph et al., 2017), so that even at a 2.5% biochar rate, all released N was possibly sequestrated by biochar. As the applied N mineralization rate was calculated by subtracting the mineral N concentration of the unamended control soil from that of the amended soils, the sequestration of mineral N in soils amended with biochar and PB1 may explain why we obtained negative applied N mineralization rates in absence of any immobilization of N. Our results are consistent with those of Ippolito et al. (2016), who observed a decrease in soil NO_3-N content with the co-application of hardwood biochar (500 °C) and manure at a 10% biochar rate. According to those authors, at 1% and 2% biochar rates, manure could have masked the effect of the biochar on decreased soil NO_3-N content by supplying sufficient inorganic N.

The mineralization of organic residues applied to soils depends on many factors, including their C/N ratio and N content (Camberato et al., 2006; Joseph et al., 2017; Rigby et al., 2016). According to Er et al. (2004), the C/N ratio of residues may explain up to 35.3% of the total variability in soil N mineralization. Rigby et al. (2016) considered the C/N ratio of organic biosolids to be the critical factor controlling the process of N mineralization in amended soils. Generally, PB with a C/N ratio < 30 produce net N mineralization within the first few weeks of

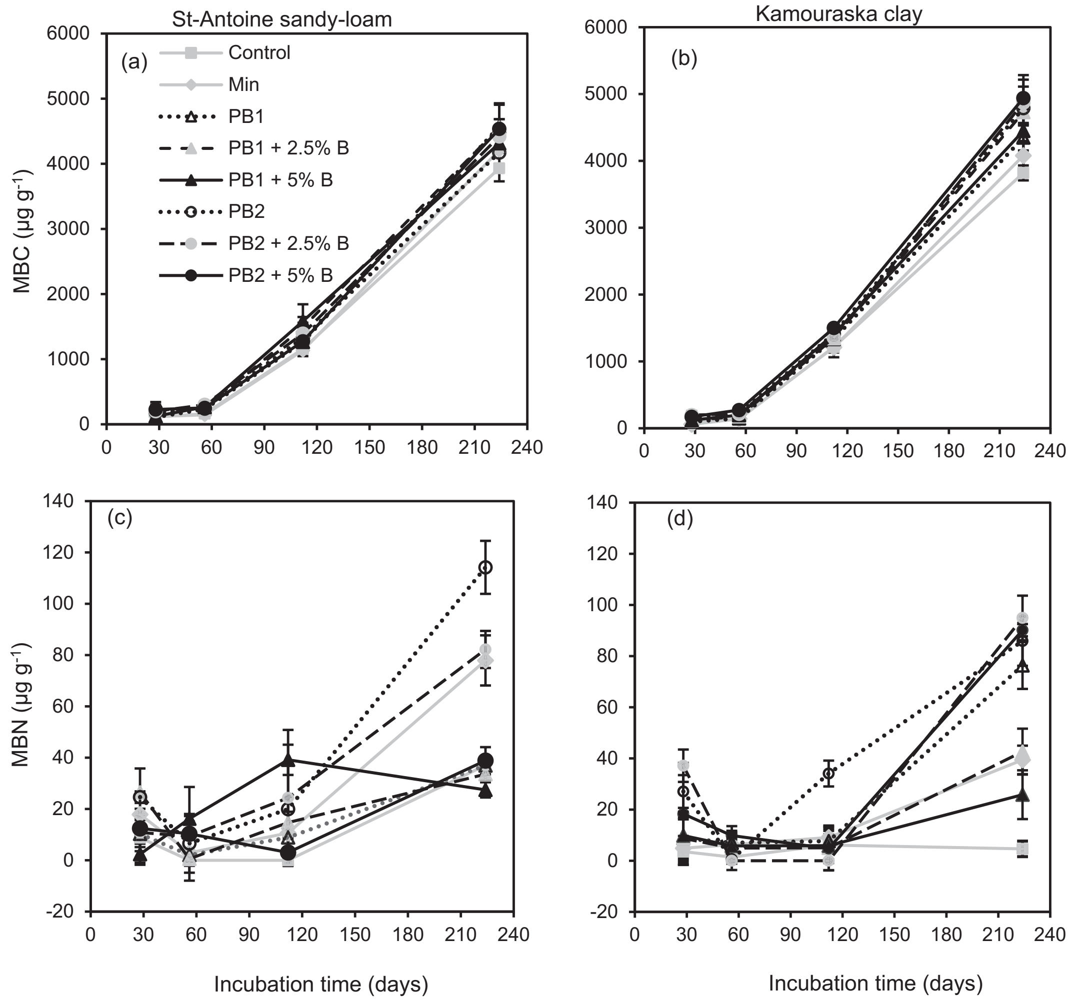

Fig. 4. Variation in soil MBC (a, b) and MBN (c, d) in St-Antoine sandy-loam and Kamouraska clay soils during a 224-day incubation. Measurement was done on days 28, 56, 112 and 224. Bars represent standard error of the mean (n = 3). Control = unamended soil, Min = mineral fertilization, PB1 + 2.5% B = PB1 + 2.5% biochar, PB1 + 5% B = PB1 + 5% biochar, PB2 + 2.5% B = PB2 + 2.5% biochar and PB2 + 5% B = PB2 + 5% biochar.

application (CRAAQ, 2010). However, in this PB category, the applied N mineralization rates are variable (Joseph et al., 2017). With high N content and a low C/N ratio (Table 2), PB2 mineralized more easily than PB1 and had a more positive impact on NH_4-N, NO_3-N, and net mineralized N concentrations and applied N mineralization rate in comparison with PB1. Our results are comparable with those of Ziadi et al. (2013), who found an increase in NO_3-N fluxes during a greenhouse study when PB with a C/N ratio of 14 were applied to soil, while some immobilization was observed when PB with a C/N ratio of 31 were used. N'Dayegamiye et al. (2004b) found that the accumulation of NO_3-N in the soil profile was lower in soil amended with PB when the C/N ratio of the material was > 20 in comparison with PB with a C/N ratio < 20.

The predominance of NH_4-N during the early stage of the incubation suggests that the nitrification process was inhibited during that stage (Dou et al., 1996). In our study, the persistence of a high soil NH_4-N concentration in the Kamouraska clay soil until day 28 seemed to be the cause of the slow increase in soil NO_3-N and net mineralized N concentrations in this soil. This situation was probably due to the low aeration conditions in the Kamouraska clay soil. According to Clough et al. (2013), the amounts of NO_3-N adsorbed by biochar depend on soil NO_3-N concentration. In our study, the decrease in the sequestration of applied N observed in the soils amended with both biochar and PB1 from day 56 in the St-Antoine sandy-loam soil seems to have been caused by a high and early release of NO_3-N in this soil. Sandy soils offer more favorable mineralization conditions, such as better aeration (N'Dayegamiye, 2009) and low NH_4-N fixation (Shah et al., 2013). Moreover, the effects of biochar on soil sorption capacity decrease over time after biochar application (Šimanský et al., 2018). Thus, as hypothesized, the impact of the co-application of biochar and PB on N dynamics depended also on incubation time in both soils.

4.3. Effect on soil microbial biomass C and N

The application of organic amendments to soils can stimulate microbial biomass through the supply of degradable C compounds and nutrients (N'Dayegamiye et al., 2004b; Tripathy et al., 2008). This explains mainly the higher soil MBC concentration observed in the soils amended with both biochar and PB in comparison with the unamended control and the soil receiving mineral fertilization. According to Lehmann et al. (2011), biochar can also influence microbial abundance through several processes, including changes in soil physicochemical properties by generating micro-habitats, which potentially provide more niches for microbial growth. This explains the increased soil MBC observed in the soils amended with both biochar and PB in comparison with those amended with PB alone. Our results are comparable to those of Cardelli et al. (2017), who reported that mixing compost (green compost and vermicompost) with biochar resulted in higher soil MBC. Similarly, Liu et al. (2016) found that the greatest positive combined effects on soil MBC were obtained when biochar amendment was coupled with composted waste N fertilizer. Li et al. (2017) observed an increase in soil MBC when the rate of biochar addition was increased, regardless of the amount of N applied. In contrast, Dempster et al. (2012) observed a decrease in soil MBC following Eucalyptus biochar co-application with wheat (*Triticum aestivum* L.) straw and composted pig manure or with inorganic N fertilizer. According to those authors, this decrease may be explained in part by the biochar feedstock. Some biomass combustion products contain toxic substances that have been specifically shown to reduce microbial activity (Zimmerman et al., 2011).

The increase in soil MBN due to the application of organic amendments indicates the stimulation of microbial N immobilization (Aoyama and Nozawa, 1993). As discussed above, the biochar used in this study contained too little labile C (Lévesque, 2017) to significantly stimulate N immobilization. Also, the PB that were used (C/N ratio < 30) produced net N mineralization. This probably explains why the addition of biochar and PB to the soils did not differently affect soil MBN in comparison to the unamended control or the mineral-fertilized soils. Our results contrasted with those of Zavalloni et al. (2011), who reported that soil MBN increased after the co-application of biochar produced from hardwood at 500 °C and wheat straw.

5. Conclusions

Our study showed that the co-application of pine chip biochar and PB greatly increased soil total C in comparison with the application of PB alone. The co-application of biochar and PB should help maintain high organic matter levels in soil. Pine chip biochar co-applied with either type of PB decreased net mineralized N concentration in both soils. The co-application of biochar with PB1, with its higher C/N ratio, drastically reduced the level of mineral N availability and led to the sequestration of released N, which can thus cause soil N deficiency. Conversely, the co-application of biochar with PB2, with its lower C/N ratio, resulted in moderate mineral N release and could therefore be interesting for agricultural soil amendment, because this method could serve as a slow N-release system with the possibility of enhancing N-use efficiency by reducing soil NO_3-N accumulation and the risk of leaching. The co-application of biochar with PB also led to higher microbial biomass in comparison with the application of PB alone, a finding that represents a great potential for the recycling of nutrients, especially N. More research under field conditions is required to confirm the benefits of the co-application of biochar and PB, particularly PB with a low C/N ratio such as PB2 in our study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.04.025.

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