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Chemical and microbiological properties of alpine forest soils: Effects of pelletized ashes in a short-term trial

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ABSTRACT

The application of wood ash to forest soils has been used to compensate nutrient loss and avoid soil and water acidification. Using ashes in a non-stabilized form might negatively affect forest ecosystems. Bearing this in mind, together with landscape restrictions and socio-economic parameters, it underlines the importance of using pelletized ashes as soil additives in alpine areas. Therefore, in this study we evaluated the effects of pelletized ashes on the chemical and microbiological properties of four soils representative for the central Alps [rendzic Leptosol (L), fibric Histosol (H), haplic Podzol (P), and dystric Cambisol (Cm)] in a microcosm trial during 22 weeks. The following ash-pellet treatments were at a ratio equivalent to 2 Mg ha^{-1}: pellets without any additive (A); or in combination with bark, compost, and digestate (B–D). A control without ashes (Ct) was also included.

Weekly measurements of pH and electrical conductivity (EC) from the soil leachates were performed. A higher pH, relative to Ct, was found in treatment A for Podsol. Additionally, treatments B and C led to a pH rise in Leptosol. Electrical conductivity, total C and N, and inorganic N forms were not significantly affected by the pelletized treatments regardless the soil type. A similar trend was recorded for pH and EC levels from the leachate samples. A lower metabolic quotient (qCO_2), relative to Ct, was recorded in Podsol following treatment A. However, when pellet ashes were applied in combination with digestate, a higher qCO_2 than that in Ct was found for this type of soil, which might indicate a lower microbial C utilization efficiency and/or microbial stress. This latter treatment also resulted in a lower dehydrogenase activity in Podsol, whilst soil protease activity and N mineralization were not affected in any of the studied soils. We conclude that ash amendment in pellets form on certain soils improves their acidity levels without causing extreme effects.

1. Introduction

Use of wood for production of heat and energy is in many places displacing fossil fuels in response to the energy targets established by the European Union (European Commission, 2010). By 2020, around 20% of the energy production is expected to be derived from renewable sources, with wooden material from forestry and forest-based industries being one of the main biomass sources in EU countries such as Finland, Lithuania, Hungary and Austria (AEBIOM, 2011). This implies the production of waste ashes, which must be properly managed to exploit their fertilizing potential while avoiding potential negative environmental impacts (Insam and Knapp, 2011; Bougnom et al., 2012; Fernández-Delgado Juárez et al., 2013; Ekvall et al., 2014).

The continuous extraction of wood and logging residues from forest ecosystems has caused a nutrient depletion, which may induce soil acidification due to a low acid neutralizing capacity (Federer et al., 1989; Glatzel, 1991; Clarke, 2012). To counteract, two different approaches can be chosen: (a) reduce harvesting intensities depending on the nutritional status of the site (Rosenberg and Jacobson, 2004; Achat et al., 2015; Pyttel et al., 2015); and (b) re-introduction of the nutrients that were extracted as a result of biomass harvesting (nutrient compensation) with the addition of wood ash (Augusto et al., 2008; Saarsalmi et al., 2012).

The potential benefits and drawbacks of using wood ash as a mineral supplement have already been tested in various studies

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(Omil et al., 2011; Saarsalmi et al., 2012; Huotari et al., 2015). It is well-known that wood ash contains major elements such as Ca, Mg, K and P, along with micronutrients including Fe, Mn, Zn and Cu, all needed for the functioning and productivity of forest ecosystems, with the exception of C and N that are mostly volatilized during the combustion process. However, the effects of wood ash may vary depending on its application rate and form (loose, hardened or granulated), as well as on the soil type (Perkiömäki and Fritze, 2002; Pitman, 2006). Along these lines, the use of loose ash has been found to induce negative effects on soil properties in the short-term, mainly due to its rapid dissolution which may lead to nutrient losses via leaching (Ozolinčius et al., 2005); and to a sharp increase in soil solution pH and salt concentration (Clapham and Zibilske, 1992; Zimmermann et al., 2002). Additionally, it may also negatively affect the populations of bryophytes and lichens (Pitman, 2006; Ozolinčius et al., 2007), as well as the soil fauna (Huotari et al., 2015), all of them playing an important role in forest nutrient cycling. It is known that soil pH regulates the magnitude of several processes, such as the solubility of metals and organic substances (Zimmermann et al., 2002). Consequently, several studies underline the benefits of using granulated or pelletized ashes in comparison with the use of loose ash (Steenari et al., 1999; Pitman, 2006; Callesen et al., 2007) in order to promote the long-lasting fertilization effect and neutralization properties of the ashes. Some studies suggest that the dissolution of granulated wood ash is delayed compared with untreated wood ash because of the increased grain size and the formation of less soluble compounds (Steenari and Lindqvist, 1997; Steenari et al., 1999; Nieminen et al., 2005). Nonetheless, there is still a gap of knowledge regarding the impact of stabilized (hardened or granulated) ash in forest systems (Huotari et al., 2015). Furthermore, most of the studies are mainly focused on the effects of stabilized ashes on plants and tree growth (Moilanen et al., 2013), rather than on the soil.

The use of ashes as a nutrient supplement in the forestry sector has a longer tradition in the Baltic and Fennoscandian countries (boreal forests) than in the central Alps. This is attributed to the fact that in the central Alps the forest is often located on very steep slopes; and secondly, due to the economical importance of tourisms, along with the "patchiness" in the alpine forests broad scale applications are uncommon. All in all this increases the difficulty of the re-circulations of nutrients from wood ash into the soil, which has not been fully embraced by the forest owners and the local communities. Therefore, the main aim of this study was to test the impact of pelletized ashes on the chemical and microbiological properties of several soils representative for the central Alps. Moreover, we investigated if such changes in soil properties derived from the use of pelletized ashes will also affect the soil-leachate chemistry.

2. Material and methods

2.1. Soil description and experimental design

For this study four representative forest soils from the Austrian Alps were chosen. Two soils developed on calcareous materials: rendzic Leptosol (L) (Terfens, 47°12′49″N 11°19′42″O) and fibric Histosol (H) (Zirl, 47°17′7″N 11°15′30″O); and two soils developed on noncalcareous materials: haplic Podzol (P) (Götzens, 47° 12′49″N 11°19′42″O) and dystric Cambisol (Cm) (Götzens, 47° 13′24″N 11°19′50″O). The sampling was performed on the 16th and 17th October, 2012. At each study site 15 soil cores (0–40 cm, Ø 11 cm) were randomly taken from a 15 × 15 m plot for the set-up of the microcosm experiment.

The laboratory-scale pellets were produced at the Institute for Material Technology (Universität Innsbruck) by using bottom ash and including the following additives at a specific ratio (ash:additive, w/w dry weight): spruce bark (75:25), sewage sludge compost (80:20) and anaerobic digested cattle slurry (95:5) (Fernández-Delgado Juárez et al., 2014). Pellets without any additive (100:0) were also used in the present study. The main properties of the pellets are shown in Table 1.

The experimental set-up was performed in Perspex columns (11 cm diameter, 40 cm depth) with a tight mesh (0.5 mm pore size) in the bottom of each column, and they were filled in with the undisturbed soil core collected *in situ* at each study site. For each type of soil, the abovementioned four ash-pellet treatments were applied on the surface of the soil column at a ratio equivalent to 2 Mg ha^{-1} of ashes according to the Austrian guidelines (Bundesministerium für Land- und Forstwirtschaft, 2011). A control treatment consisting of the soil core without addition of any pellet (Ct) was also included. A total of 60 experimental units (5 amendments × 4 soil types × 3 replicates) were set-up. After an equilibration period of 4 days at 4 °C, all columns were randomly placed into an incubation chamber at 8 °C (that is the average temperature for the months of October–November in the study areas) with a light/darkness cycle of 8/16. Soil cores were weekly watered with distilled water, considering the average rainfall regime (970–1500 mm/year rainfall) of the autumn season from

Table 1
Nutrient and heavy metal content of the pellets used in this study, including the limit values for quality ashes A and B according to the Austrian Guidelines for Ash Use (Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasser, 2011). Values are expressed on a dry weight (dw) basis. Values are means with the standard error (SE) in brackets (n = 3).

Limits for different ash qualities			Nutrient and heavy metal contents			
	A	B	Pellet-ash (A)	Pellet-bark (B)	Pellet-compost (C)	Pellet-digestate (D)
pH	–	–	10.9 (0.1)	10.0 (0.1)	10.7 (0.1)	11.7 (0.1)
EC (mS cm^{-1})	–	–	0.78 (0.02)	0.66 (0.01)	1.24 (0.04)	1.33 (0.04)
VS (g kg^{-1})	–	–	103 (2.3)	220 (0.8)	123 (1.7)	54.3 (0.8)
C (g kg^{-1})	–	–	38.5 (1.6)	121 (4.2)	73.1 (3.0)	36.8 (3.1)
N (g kg^{-1})	–	–	0.25 (0.04)	1.65 (0.24)	4.69 (0.23)	0.52 (0.03)
Ca (g kg^{-1})	–	–	77.1 (1.94)	74.2 (1.45)	77.4 (4.09)	88.1 (0.89)
Mg (g kg^{-1})	–	–	36.4 (0.61)	26.7 (0.79)	26.8 (1.25)	30.1 (1.47)
K (g kg^{-1})	–	–	37.4 (0.54)	29.6 (0.41)	34.9 (0.51)	34.5 (0.24)
As (mg kg^{-1})	20	20	0 (0)	0.72 (0.46)	1.33 (0.80)	1.11 (0.77)
Zn (mg kg^{-1})	1200	1500	1.56 (0.11)	2.98 (0.18)	6.07 (0.44)	2.32 (0.20)
Cu (mg kg^{-1})	200	250	1.58 (0.12)	3.45 (0.15)	4.92 (0.29)	1.55 (0.08)
Cr (mg kg^{-1})	150	250	10.3 (0.59)	11.0 (0.69)	11.0 (1.18)	9.00 (0.31)
Pb (mg kg^{-1})	100	200	2.66 (0.70)	1.06 (0.3)	1.80 (0.06)	0.60 (0.21)
Ni (mg kg^{-1})	150	200	3.47 (0.12)	2.91 (0.19)	3.52 (0.23)	2.72 (0.21)
Cd (mg kg^{-1})	5	8	0 (0)	0 (0)	0 (0)	0 (0)

each of the study areas since the last 20 years. Soil leachate was sampled weekly for determination of pH and electrical conductivity (EC). All the columns were destructively sampled after 22 weeks in order to determine the effects of the different pellet treatments on the soil parameters described below. Prior to sieving (<2 mm), soil columns were divided into two parts, consisting of an upper layer (0–10 cm) and a lower layer (10–40 cm). Only the upper layer (0–10 cm) was analyzed in this study. The topsoil is considered biologically more active (Ozolinčius et al., 2005; Chodak et al., 2015) and more responsive to ash addition. Samples were then sieved (<2 mm), carefully separated from root fragments and stones, and kept at 4 °C for physico-chemical and biological analyses and at −20 °C for enzymatic analyses, respectively. Biological parameters were measured within 5 days.

2.2. Soil analyses

Soil samples (10 g, fresh weight) were oven-dried (105 °C) for 24 h, and re-weighed for calculating total solids. The volatile solids (VS) content was determined from the weight loss following ignition in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Total C and N were analyzed in dried samples, using a CN analyzer (TruSpec CHN; LECO, Michigan, U.S.A.). Electrical conductivity and pH were determined in distilled water and 0.01 M $CaCl_2$ extracts (10:25, w/v), respectively. Ammonium and nitrate were determined according to Kandeler (1993c,b). Nutrient and heavy metal contents in soil samples were determined after digestion with nitric acid using a microwave system Speedwave 4 (Berghof, Eningen, Germany), followed by a GF-AAS contrAA® 600 (Analytic Jena, Jena, Germany).

Basal respiration (BR) and microbial biomass (C_{mic}) were measured according to Heinemeyer et al. (1989). The metabolic quotient (qCO_2, μg CO_2–C g^{-1} C_{mic} h^{-1}) was calculated from R_{mic} and C_{mic} according to Anderson and Domsch (1989). For the determination of the ammonification (AM) activity, soil samples were saturated with water for 7 days, and the ammonium released was measured according to Kandeler (1993a). Dehydrogenase activity (DHA) was photometrically determined following the procedure of Öhlinger (1993). Protease activity (PA) was colorimetrically assessed according to Ladd and Butler (1972).

2.3. Leaching trial

Leachate pH and EC values were determined using a pH-meter (MLP4, WTW, Weilheim, Germany), and a LF 330 conductivity meter with a standard cell TetraCon 325 (WTW, Weilheim, Germany), respectively, immediately after sampling,

2.4. Statistical analyses

To test the effects of the pellet treatments on the aforementioned soil parameters, data were subjected to a one-way ANOVA. When significant *F*-values were obtained, further analysis with Tukey's HSD (honestly significant difference) comparison procedure was performed as a *post hoc* test ($p < 0.05$). Data were tested for normality and transformed when necessary. Leaching data were analyzed by repeated measures analysis of variance (ANOVAR) in which soil columns represented the subjects, pellet treatment was fixed as the between-subject factor, and the sampling time was fixed as a within-subject factor. In case that the variables did not meet the sphericity condition (Mauchly's test), the sphericity violation was corrected with the Geisser–Greenhouse (G–G) procedure (Potvin et al., 1990). All the analyses were performed with the Statistica software (version 9).

3. Results and discussion

One of the main effects of using wood ash as a soil fertilizer relies on its known capability to buffer pH (Pitman, 2006; Insam and Knapp, 2011; Huotari et al., 2015). Moreover, pH is considered as a determinant factor in soil, because it has been shown to affect the nutrient availability and uptake, as well as the biomass, activity, and composition of the microbial community (Demeyer et al., 2001; Rousk et al., 2009). In our study, the pH was significantly increased by the addition of pellet ashes in Podsol (P) and Leptosol (L) soils (ANOVA $F_{4,10} = 4.48$, $p < 0.05$; ANOVA $F_{4,10} = 4.64$, $p < 0.05$, for P and L soils respectively). Specifically, in the case of Podsol the pH value was around 0.5 units higher in treatment A (100% pellet ashes) than in the control (Table 2). The use of additives in combination with pelletized ashes increased the pH in Podsol slightly to the control, but these differences were

Table 2
pH, electrical conductivity (EC), volatile solids (VS), C_{tot}, N_{tot}, *C*/*N* ratio, ammonium and nitrate content of the ash-treated soils (Ct: Control, A: Ash pellet, B: Ash/Bark pellet, C: Ash/Compost pellet, D: Ash/Digestate pellet) after 22 weeks. Values expressed on a dry weight basis for $n = 3$ (SE in brackets).

Soil	Treatment	pH	EC mS cm^{-1}	VS mg g^{-1}	C_{tot} mg g^{-1}	N_{tot} mg g^{-1}	*C*/*N* ratio	NH_4^+ μg g^{-1}	NO_3^- μg g^{-1}
Podsol	Ct	3.77 (0.06) a	114 (12.1)	804 (41.7)	461 (24.1)	16.3 (0.43)	28.3 (0.79)	242 (31.8)	42.9 (9.60)
	A	4.23 (0.10) b	83.6 (6.9)	710 (45.7)	449 (15.9)	15.5 (0.48)	29.0 (0.89)	404 (200.4)	27.0 (1.66)
	B	4.19 (0.09) ab	109 (8.6)	728 (77.1)	454 (27.9)	15.8 (0.15)	28.7 (2.11)	281 (28.6)	32.2 (3.26)
	C	3.97 (0.05) ab	109 (14.0)	755 (80.3)	495 (5.0)	16.8 (1.62)	30.0 (2.84)	295 (52.6)	39.5 (2.66)
	D	4.18 (0.13) ab	98.4 (13.7)	862 (22.5)	432 (24.3)	16.3 (3.30)	28.7 (5.52)	298 (92.9)	23.0 (5.13)
Cambisol	Ct	4.81 (0.02)	81.6 (24.4)	284 (47.2)	233 (57.1)	12.1 (1.94)	18.9 (1.63)	176 (50.7)	10.7 (4.25)
	A	5.08 (0.14)	104 (34.6)	223 (15.6)	166 (32.3)	7.61 (0.97)	21.4 (1.55)	122 (27.7)	20.6 (8.75)
	B	5.25 (0.13)	100 (23.5)	315 (87.5)	219 (51.2)	9.72 (1.96)	22.2 (1.48)	164 (26.0)	29.2 (15.7)
	C	5.21 (0.24)	134 (37.5)	461 (115.2)	170 (40.1)	8.74 (1.34)	20.3 (0.85)	126 (40.5)	34.6 (10.1)
	D	4.90 (0.24)	118 (46.5)	212 (6.6)	145 (1.3)	7.17 (0.29)	19.0 (2.14)	89.8 (15.3)	36.0 (8.32)
Histosol	Ct	7.06 (0.22)	224 (46.6)	489 (70.0)	288 (33.9)	8.63 (0.60)	33.2 (1.61)	119 (9.5)	9.53 (2.62)
	A	6.54 (0.14)	161 (16.5)	503 (13.8)	312 (17.1)	8.53 (0.48)	36.9 (3.22)	111 (14.1)	12.7 (1.70)
	B	6.89 (0.04)	242 (43.5)	485 (93.2)	302 (42.3)	9.81 (0.78)	30.6 (2.84)	84.8 (4.0)	12.4 (2.72)
	C	6.90 (0.42)	223 (37.5)	492 (117.4)	303 (71.2)	11.04 (2.23)	27.0 (1.27)	117 (18.5)	18.3 (10.9)
	D	7.07 (0.22)	260 (51.9)	456 (63.02)	274 (26.4)	8.39 (0.50)	32.8 (3.29)	133 (6.5)	8.20 (2.34)
Leptosol	Ct	6.52 (0.16) a	135 (14.6)	224 (19.0)	121 (14.5)	7.18 (0.78)	16.9 (0.40)	2.58 (0.53)	61.1 (6.08)
	A	7.20 (0.19) ab	176 (19.6)	271 (30.1)	148 (19.6)	9.04 (0.07)	16.4 (2.3)	1.83 (0.14)	60.8 (15.6)
	B	7.31 (0.04) b	185 (11.2)	264 (49.0)	151 (33.8)	9.41 (0.40)	15.8 (2.82)	1.78 (0.01)	80.0 (12.5)
	C	7.28 (0.16) b	176 (20.6)	186 (11.3)	93.4 (9.8)	7.35 (0.62)	12.7 (0.44)	2.15 (0.05)	50.3 (6.82)
	D	7.02 (0.15) ab	159 (6.6)	232 (19.8)	126 (8.6)	7.81 (0.27)	16.1 (0.54)	1.88 (0.12)	56.4 (6.82)

Dissimilar letters in a column indicate statistically significant differences among the ash treatments (Tukey HSD-test).

not statistically significant (Table 2). However, no significant changes were found in pH for Cambisol following pellet addition, despite also being a noncalcareous soil (Table 2). Concerning the two calcareous soils, an increase in pH was only observed for Leptosol (ANOVA $F_{4,10} = 4.64$, $p < 0.05$), being around 0.8 units higher in treatments B and C (ash pellets in combination with spruce bark and sewage sludge compost, respectively) than in the control (Table 2). Despite the fact that organic soils are expected to be more influenced by ash addition than mineral soils, an increase in pH was not recorded for Histosol. In fact, and unexpectedly, the treatment A presented lower pH values (6.54) than Ct (7.06) despite the high pH value (10.9) of the added pellets; however, these differences were not statistically significant. Such findings could be probably due to the presence of a thick humus layer and/or to the inherent heterogeneity of forest soils (Bruckner et al., 1999). According to Rosenberg et al. (2010), the addition of ashes in form of pellets or granules need not always be accompanied by a pH rise because the pelletized ashes dissolve gradually into the soil, thereby avoiding an abrupt pH increase. These authors also underlined that the effects of granulated ashes on the pH of humus soil layers are greatly influenced by N deposition or the N status of the site where the ash is applied.

The pH of the leachate was not affected by the addition of pelletized ashes (Fig. 1). Nonetheless, for Leptosol a significant pH increase was found over time (ANOVAR $F_{18,180} = 19.89$, $p < 0.001$). Indeed wood ash addition to forests was primarily considered as a strategy to reduce acid waters leading to an increase in the pH of the water stream (Meiwes, 1995). However, Norström et al. (2012) did not find an effect of ash addition on the pH of the soil solution from both Arenosol and Podsol in a 4-year study. On the other side, Piirainen et al. (2013) found that the addition of hardened ash evoked an increase in peatland runoff pH during a 10-year trial. This increase was, nevertheless noticeable only for 3–4 years in some of the study sites, and for the whole study period in others.

Another important parameter to evaluate in ash-amended soils is the EC, an indicator for soil salinity (Clapham and Zibilske, 1992; Perucci et al., 2008). EC was not significantly affected by any of the pellet treatments irrespective of the type of soil (Table 2). For reasons of plant health, soil conductivity should not exceed the threshold value of 2 dS m^{-1} (Herrero and Pérez-Coveta, 2005), a limit that was never reached in this study following the application of pelletized ash. Leachate samples EC was not significantly influenced by any of the ash pellet treatments (Fig. 2). However, significant changes were recorded in this parameter over time for all the soils, except for Podsol (Fig. 2). For L, Cm and H an increase in leachate EC was observed during this trial. Nevertheless for soils developed in calcareous materials L and H, on week 17 of the trial there was a sharp decrease in the values, after which value stabilized again. This pattern might be attributed to temporal differences in ions solubilization (Pitman, 2006; Huotari et al., 2015), even though such effects have been primarily observed in peatlands and non-calcareous soils. Núñez-Delgado et al. (2011) reported a slight increase of runoff EC after the addition of loose ash into a mineral acid soil for a period of 4 months after ash addition. Along these lines, Piirainen et al. (2013) also observed an

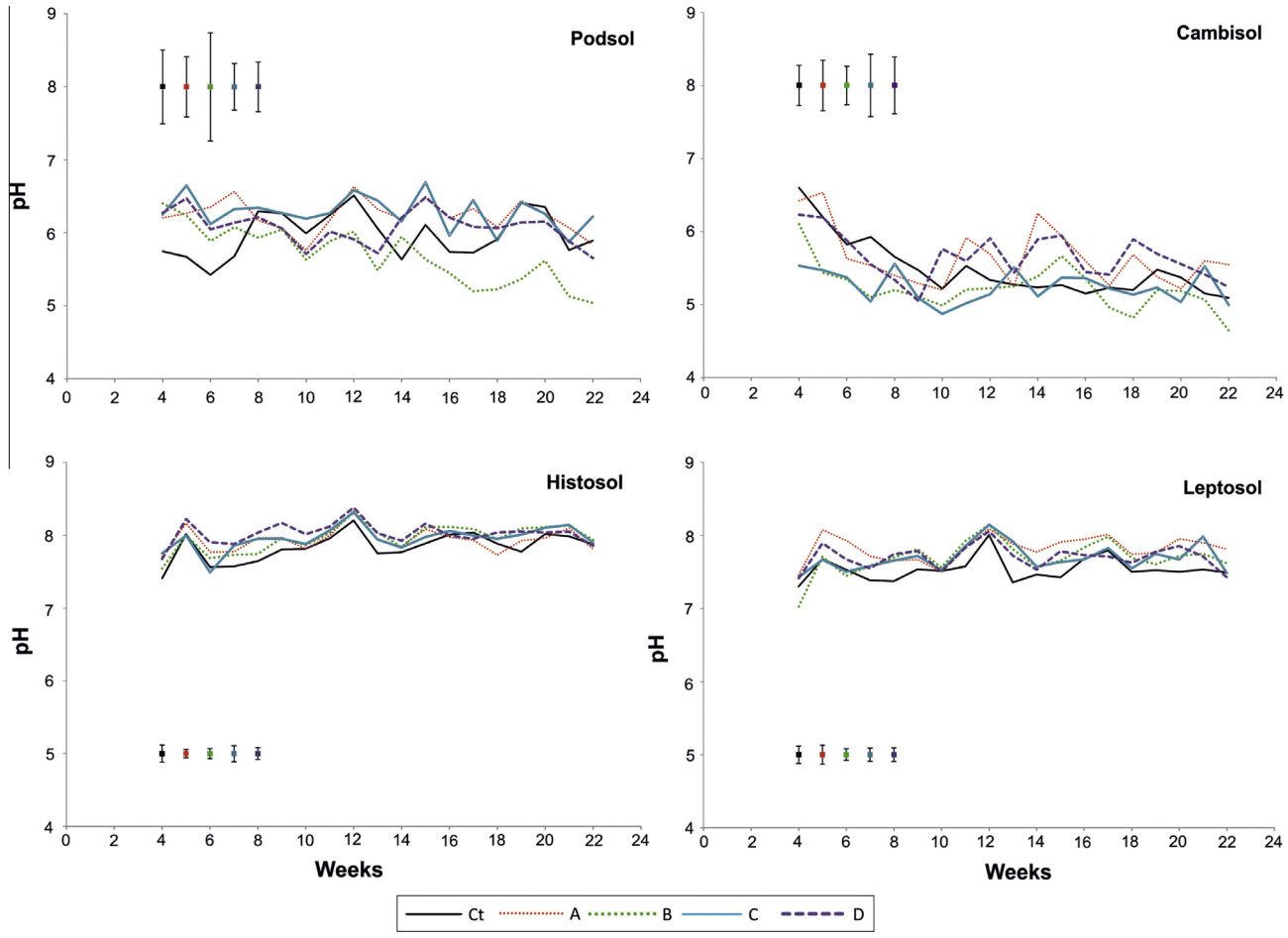

Fig. 1. Dynamics of leachate pH of the ash-treated soils (Ct: control, A: ash pellet, B: ash/bark pellets, C: ash/compost Pellet, D: ash/digestate pellet) during 22 weeks. Means of triplicate measurements, bars indicate average s.e. for each treatment.

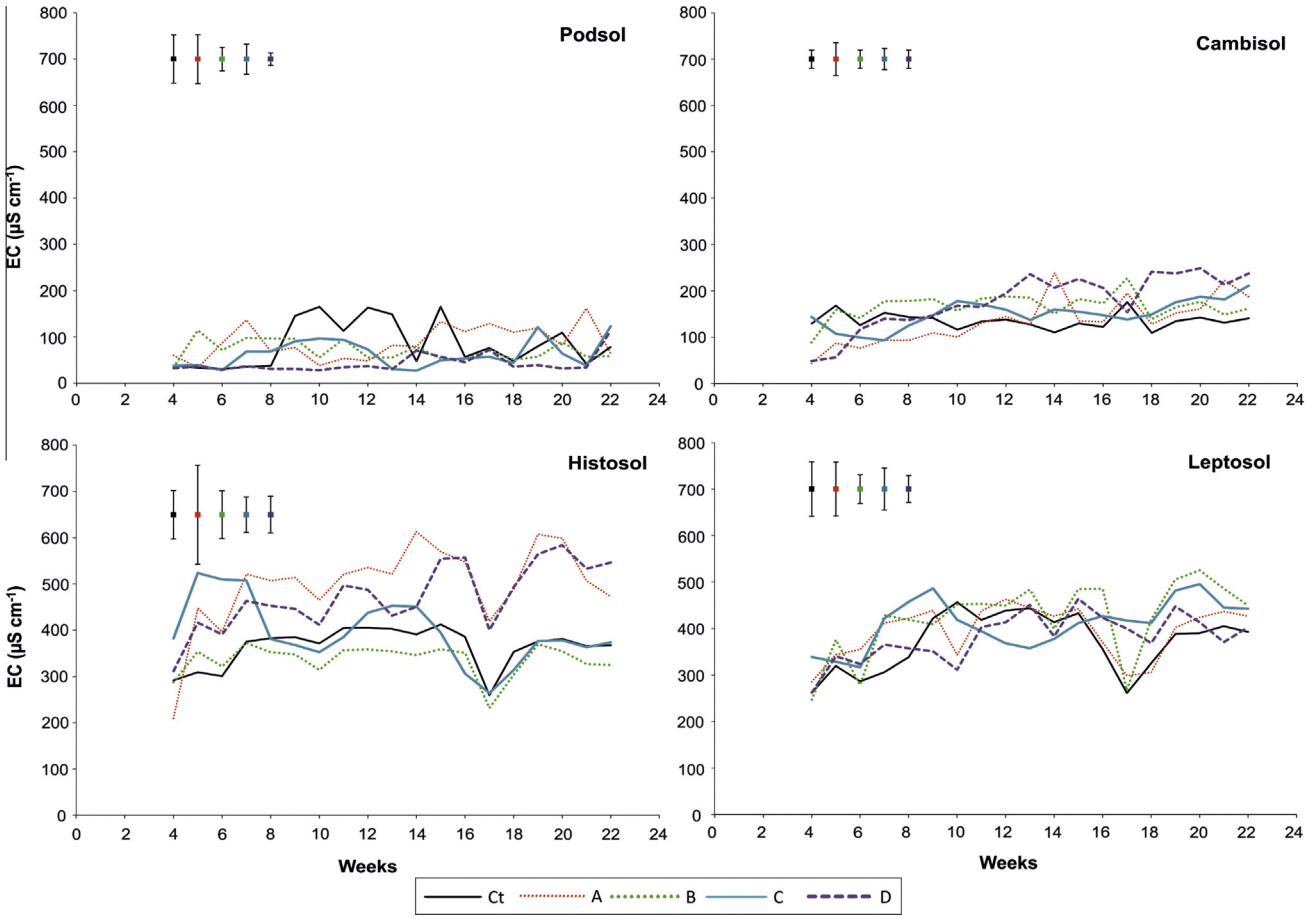

Fig. 2. Dynamics of leachate electrical conductivity of the ash-treated soils (Ct: control, A: ash pellet, B: ash/bark pellets, C: ash/compost Pellet, D: ash/digestate pellet) during 22 weeks. Means of triplicate measurements, bars indicate average s.e. for each treatment.

increase in the abovementioned parameter after the first year of having added ashes in three different study sites (drained peatland forests) during a 10-year trial and in all of them an increase in runoff EC compared to the control was found, one year after ash addition, but not in the successive years. Such increase in runoff EC was recorded only for some treatments and non-clear distinction between loose and granulated ash was observed.

For the remaining soil chemical parameters measured in the present study (i.e., C_{tot}, N_{tot}, C/N ratio, NH_4^+, NO_3^-) no significant treatment effects were found (Table 2). Moreover, in the present study heavy metals contents in all the soil types and regardless the treatment (data not shown), were below the levels that define a soil as a contaminated site according to German and Swiss regulations (Bundesgesetztblatt, 2012; Der Schweizerische Bundesrat, 2012). It has been shown that ash addition induces the mineralization process in the soil, which may favor the production of organic-N compounds, and ultimately result in an increase of inorganic N forms (Saarsalmi et al., 2012; Huotari et al., 2015). However, according to Rosenberg et al. (2010), N mineralization processes do not seem to be affected by ash addition when applied at N-poor sites. These authors did not find a change neither in N pools nor in C_{tot} and N_{tot} concentrations in soils amended with up to 6 Mg granulated ash ha^{-1}. In addition, Moilanen et al. (2013) did not find significant differences in the C/N ratio in peatlands and sandy mineral uplands after addition of self-hardened and granulated ash for 13 years. These results are confirmed by our observations.

Soil biological properties were found to be more sensitive when evaluating the impact assessment after application of fertilizers due to the fact that changes in microbial communities may occur more quickly than changes in other soil properties (Doran and Zeiss, 2000; Abubaker et al., 2013). Nevertheless, in our study soil microbial biomass and activity assessed by C_{mic} and BR, respectively were not affected by any of the pellet treatments independent of whether the soil was calcareous or not (Table 3). Also, Björk et al. (2010) found that crushed ash addition did not have a significant effect on BR and SIR in three different peatlands after a period of 1, 4 and 25 years. However, when microbial biomass was assessed by the PLFA technique, these authors found a decrease even after 4 years since ash amendment in an oligotrophic peatland; whereas, no changes were observed in a mesotrophic site that was richer in nutrients. On the other hand, Zimmermann and Frey (2002) reported an increase in both BR and C_{mic} after adding 8 Mg ha^{-1} of loose ash to a Cambisol in a mixed forest. In the present study, despite the lack of differences in microbial biomass and soil respiration, a lower qCO_2 relative to the control without any additive was recorded in Podsol for treatment A (ANOVA $F_{4,10} = 4.52$, $p < 0.05$). No significant effects were recorded for treatments B and C (Table 3). In contrast, a higher qCO_2 than that in the control was found for treatment D, which is the addition of pellet ashes in combination with digestate (Table 3). The metabolic quotient is an indicator of microbial C utilization efficiency and its increase may indicate microbial stress (Anderson and Domsch, 1993; Wardle and Ghani, 1995). Therefore, the abovementioned findings might be indicative of an enhancement of the C use from the Podsol microbial community, probably due to an increased C mineralization following the addition of pelletized ashes without any additive (Perkiömäki et al.,

Table 3
Biological properties:basal respiration BR, microbial biomass C_{mic}, metabolic quotient qCO_2, microbial quotient C_{mic}/C_{org}, and enzymatic activities of the ash-treated soils (Ct: Control, A: Ash pellet, B: Ash/Bark pellet, C: Ash/Compost pellet, D: Ash/Digestate pellet) after 22 weeks Values expressed on a dw basis for $n = 3$ (SE).

Soil	Treatment	BR μg CO_2 Cg^{-1} soil h^{-1}	C_{mic} μg C g^{-1} soil	qCO_2 μg CO_2-C mg^{-1} C h^{-1}	C_{mic}/C_{org} (%)	Dehydrogenase μg TPF g^{-1} soil 16 h^{-1}	Protease μg Tyrosine-eq g^{-1} soil 2 h^{-1}	N-mineralization μg N g $soil^{-1}$ d^{-1}
Podsol	Ct	22.1 (2.18)	2216 (269)	10.5 (0.28) ab	0.45 (0.04)	2100 (384) a	1116 (161)	75.8 (7.76)
	A	17.6 (2.44)	2012 (201)	8.69 (0.39) a	0.49 (0.06)	1220 (251) ab	1245 (213)	98.9 (25.3)
	B	30.0 (4.68)	2532 (197)	11.8 (1.03) ab	0.60 (0.04)	1003 (236) ab	981 (106)	81.4 (14.1)
	C	29.7 (2.92)	2311 (99.2)	10.7 (0.52) ab	0.46 (0.03)	1035 (202) ab	1113 (65.6)	66.7 (13.5)
	D	24.1 (2.51)	2277 (323)	12.8 (1.05) b	0.52 (0.03)	664 (99.3) b	1109 (160)	78.1 (13.9)
Cambisol	Ct	11.2 (4.96)	1142 (411)	9.39 (0.88)	0.65 (0.13)	930 (85.1)	980 (394)	39.1 (8.80)
	A	9.38 (2.79)	1055 (262)	8.71 (0.40)	0.80 (0.14)	1720 (146)	800 (222)	34.7 (6.97)
	B	18.1 (4.16)	1726 (419)	10.5 (0.42)	0.96 (0.08)	1810 (413)	889 (204)	42.6 (11.8)
	C	16.8 (7.40)	1835 (755)	8.66 (0.48)	0.81 (0.34)	1365 (264)	1185 (378)	53.8 (16.7)
	D	6.93 (0.54)	813 (87.8)	8.60 (0.46)	0.66 (0.07)	1420 (63.0)	504 (7.43)	31.7 (4.30)
Histosol	Ct	31.2 (5.46)	3045 (461)	10.2 (0.25)	1.08 (0.11)	22,180 (4730)	1490 (257)	36.4 (7.05)
	A	34.9 (3.26)	3338 (185)	10.4 (0.71)	1.14 (0.07)	16,110 (2180)	1494 (132)	33.8 (1.61)
	B	33.1 (2.18)	3315 (445)	10.3 (1.24)	1.27 (0.29)	22,160 (3320)	1480 (166)	34.9 (7.95)
	C	32.2 (5.88)	3765 (778)	8.68 (0.41)	1.34 (0.06)	20,270 (6230)	1660 (296)	41.9 (3.94)
	D	43.5 (4.72)	3950 (304)	11.1 (1.40)	1.52 (0.11)	22,997 (1900)	1348 (206)	37.1 (1.51)
Leptosol	Ct	9.79 (1.77)	1278 (182)	7.56 (0.39)	0.97 (0.07)	6220 (1080)	1538 (88.0)	20.5 (2.79)
	A	15.9 (4.07)	1950 (280)	7.89 (0.84)	1.23 (0.06)	9900 (2790)	1893 (287)	25.2 (4.10)
	B	14.1 (5.53)	1778 (577)	7.59 (0.58)	1.10 (0.15)	11,560 (3070)	2088 (280)	25.0 (9.98)
	C	8.56 (1.62)	1440 (98.9)	5.89 (0.94)	1.34 (0.14)	7500 (811)	1585 (202)	18.3 (1.90)
	D	10.51 (0.86)	1270 (119)	8.41 (0.95)	0.94 (0.06)	7350 (1206)	1492 (236)	21.4 (4.72)

Dissimilar letters in a column indicate statistically significant differences among the ash treatments (Tukey HSD-test).

2004), this increase can be defined as a positive priming effect (Kuzyakov et al., 2000). For the remaining soils, there were no significant differences among the treatments regarding qCO_2 (Table 3). This reinforces the harmless action of pelletized ashes on the soil microbiota (Perkiömäki and Fritze, 2002; Huotari et al., 2015).

Dehydrogenase activity (DHA) is a measurement of intracellular activity reflecting the metabolic ability of the soil. This enzymatic activity is considered to be proportional to the biomass of the microorganisms in soil, and it is often dependent on soil pH, moisture and organic matter content (Wolińska and Stępniewska, 2012). In the present study, a significant reduction in Podsol DHA, relative to Ct, was recorded when pelletized ashes were amended with digestate (ANOVA $F_{4,10} = 14.60$, $p < 0.05$, Table 3). Nonetheless, for the other three soils no significant changes were observed among the different pellet treatments (Table 3). As shown in Table 2, the lowest pH value was recorded in Podsol which could be a plausible explanation of why the biological properties from Podsol (i.e. qCO_2 and DHA) are more sensitive to ash addition, since changes in soil microbial communities are often related to a change in soil pH (Huotari et al., 2015).

According to Zimmermann and Frey (2002), the lack of N in the ashes together with the boost of microbial activity might be accompanied by an increased demand for available N-compounds, and in turn by a stimulation of the enzymes related to the N-cycle. Additionally, organic N forms were found to act as a primary source of N in boreal forests with developed organic horizons (Kielland et al., 2007). All in all this underlines the importance of measuring the protease activity in ash-amended soils. However, in our study this enzymatic activity along with N-mineralization were not significantly affected by any of the pellet treatments independent of whether the soil was calcareous or not (Table 3). This is in accordance with the trends observed for both BR and C_{mic}.

4. Conclusions

Overall, Podsol was found to be more sensitive to ash addition than the other three types of alpine soils. Of interest is that the application of digestate in combination with pelletized ashes can exert a harmful action on Podsol soil microbiota, as indicated by the higher qCO_2 and lower dehydrogenase activity. However, the other two additives (i.e. spruce bark and composted sewage sludge) affected the soil properties in a similar way than did the control treatment and/or when the pelletized ashes were applied without any additive, regardless the soil type. Although the present findings should not be extrapolated to all soil types and field conditions, it is expected that the current study adds further evidence as to the potential value of using pelletized ashes as soil additives in forests. Moreover, we need to constrain our conclusions to the short-term effects and further trials in the long-term would help to achieve a more comprehensive picture of the effects of pelletized ash on alpine soils in the long-term.

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