Biol Fertil Soils (2013) 49:575–585
DOI 10.1007/s00374-012-0747-5

ORIGINAL PAPER

Wood ash effects on chemical and microbiological properties of digestate- and manure-amended soils

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Received: 17 July 2012 / Revised: 9 October 2012 / Accepted: 17 October 2012 / Published online: 30 October 2012

Abstract A field study was carried out to evaluate the potential of wood ash as a fertilizer in grassland systems in combination with enriched N organic wastes. Six treatments including manure or digestate, each combined with wood ash at 0, 1, and 3 tha^{-1} were spread onto the soil to an amount equivalent to 120 kgN ha^{-1}. Three soil samplings and one cutting was carried out within one growing season (3 months). A higher pH value was found in manure-treated plots, the pH rise being proportional to the amount of wood ash added. Those plots amended with digestate were characterized by a larger content of total C, NH_4^+, and total P (TP) regardless of the amount of ashes. Microbial activity, assessed by basal respiration and microbial biomass carbon of the differently treated soils, was not affected neither by the nature of the organic waste nor by the amount of wood ash added. However, amending soil with digestate resulted in a more efficient soil microbial community, as shown by the lower values of the metabolic quotient. Such effects were accompanied by a higher percentage of plant cover, particularly of leguminous plants in digestate-treated plots. The time of sampling (seasonal effects) was found to influence the soil pH and electrical conductivity (EC), as well as the nutrient content (total N, NH_4^+, and NO_3^-). Overall, the combined use of wood ash and biogas digestate can constitute an efficient way for the disposal and recycling of both products and additionally, it may constitute an environmentally friendly alternative to mineral fertilizers for acid soils.

Keywords Renewable energy resources · Cattle manure · Grasslands · Inorganic P · Metabolic quotient · Plant cover

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Introduction

Renewable energy is becoming increasingly important in times of decreasing fossil fuel supplies (European Commission 2010). In this context, biomass combustion in centralized heat and power plants is gaining popularity and is producing huge amount of ashes (Knapp and Insam 2011). For instance, only in Austria, the quantities of wood and straw ash increased from 100,000 t in 2004 to 149,000 t in 2008 and its disposal in landfills is still a common practice (Umweltbundesamt GmbH 2011). The increasing costs for biomass plant operators along with the difficulties of acquiring new landfill sites have, thus, encouraged the search for alternative methods of management in order to exploit the recycling potential of ashes.

It has been reported that the application of wood ash, with pH values ranging from 8 to 13 (Augusto et al. 2008) has a high acid-neutralizing capacity in soils due to the formation of hydroxides and carbonates during the combustion and conditioning processes (Steenari et al. 1999). Such an increase in pH is expected to affect the solubility of the elements in soil (Augusto et al. 2008), as well as to increase the soil cation exchange capacity and base saturation (Saarsalmi et al. 2001, 2004). In addition, wood ash is essentially a direct source of either major elements such as P, Ca, Mg, and K, with the exception of C and N that are mostly volatilized during combustion (Odlare and Pell 2009). Various trace elements including Fe, Mn, Zn, and Cu can also be present in the ash, thereby reinforcing its ameliorative effects in soil (Kuba et al. 2008). Indeed, previous studies have highlighted that ashes act as mineral fertilizers and lime (Holzner 1999; Pitman 2006); however, Demeyer et al. (2001) reported negligible or negative effects of ashes in case of N limitations in soils, suggesting balancing ash amendments with N fertilization. This fact has also been supported by Saarsalmi et al. (2006), who found a long-term effect only for combination treatments with N fertilizer. Therefore, the application of ashes with N-enriched organic wastes such as anaerobic slurries, another by-product of renewable energy production, may result in additional benefits, particularly for acid soils, as their nutrient contents complement each other (Bougnom and Insam 2009; Bougnom et al. 2010). Recently, Massé et al. (2011) reported that the enrichment of mineral fractions of N and P during the anaerobic digestion of manure increased the concentration of plant-available nutrients and subsequently, promoted plant growth (Bougnom et al. 2012). Nevertheless, the use of anaerobically digested manure, termed digestate in the text below, as an organic amendment should accurately match crop N demand because if not taken up by the plants, nitrates could be drained to surface waters, leached to ground waters, or be denitrified into gaseous forms and emitted to the atmosphere (Insam and Merschak 1997). Additionally, cattle manure digestates usually have a pH value close to 8, which is around 0.5 U higher than that of the untreated manure (Bougnom and Insam 2009), indicating that if combined with ash it could lead to a larger increase in the slurry pH (Bougnom et al. 2012), thereby favoring gaseous loss of ammonia and affecting the solubility and the availability of trace elements in soil (Demeyer et al. 2001). The effects of wood ash are primarily governed by soil type and application rate (Pitman 2006). These latter authors established that the benefits of using wood ash in forestry are maximized at low dose rates, with a possible toxicity from applications in excess of 10 tha^{-1}. In line with this, other studies have shown different trends in the microbiological properties of ash-treated soils regarding the dose of application. For instance, Gering et al. (2000) observed a slight increase in the microbial biomass C of a forest soil after 15 months of treatment with 4.8 tha^{-1} of wood ash; in contrast, Zimmermann and Frey (2002) found a rapid increase in this parameter followed by a decrease 62 days later after the application of 8 tha^{-1} of wood ash in a forest soil. Perucci et al. (2008) found that a higher rate of ash addition (20 tha^{-1}) had a more pronounced effect on the chemical and microbiological soil properties than at lower rates (5 tha^{-1}) on an agricultural system, although such effects disappeared 12 months after the treatment. All of the above indicates that the rate of application of ashes is a crucial factor to be considered when they are used as fertilizers in both agricultural and forest soils.

Overall, soils in Alpine grasslands are highly acidic and of low fertility and as such, most of these mountain areas require the application of a liming agent to bring them to their optimum pH, as well as to compensate for their nutrient deficiencies, especially for P and K (Ferreiro et al. 2011). Considering the above-mentioned properties of wood ash, its use appears to be a valid and less expensive option for the management of this type of areas, thereby contributing to an integrated environmental strategy for the recovery of marginal land and to promote the sustainable use of ashes as soil fertilizers. Additionally, the on-farm production of renewable energy by anaerobic digestion has increased over the last few years (Insam and Wett 2008; Holm-Nielsen et al. 2009), and consequently, biogas residues accrue in large quantities and their land application has become a widespread agricultural practice (Weiland 2003). As such, it is still necessary to delve deeper into the potential benefits and drawbacks of digestate on soil from both a chemical and microbiological viewpoints in order to guarantee its safe use for the environment and highlight its use as a sustainable alternative to manures for the maintenance of plant health and soil fertility. In fact, legislative frameworks focused on digestate quality management are still scarce in Austria compared with those related to well-recognized products such as composts and biosolids (Al Seadi and Lukerhurst 2012). Furthermore, up to now, there is still a lack of information regarding the combined use of both by-products of renewable energy production, that is, wood ash and digestate on grassland soils (Bougnom et al. 2012), and the major factors (i.e., dose of application) driving such effects (Ferreiro et al. 2011). Therefore, the aim of the present study is to evaluate the impact of wood ash dose on the chemical and microbiological properties of a grassland system to gain further insight of its potential as a soil fertilizer in combination with either untreated or digested manure. Moreover, we investigated if changes in soil properties would affect the total plant cover as well as the fractions of leguminous plants and grasses.

Specifically, we determined the changes in pH, electrical conductivity (EC), as well as in nutrient content (C, N, and P) of the differently treated soils over time. pH is considered one of the most determinant factors in soil (Smith and Doran 1996) as it can affect the nutrient availability and uptake, along with the biomass, activity, and composition of the microbial community (Demeyer et al. 2001; Rousk et al. 2009). The EC is also an important parameter to evaluate in soils because it reflects the degree of salinity and suitability for crop growth (Smith and Doran 1996). We also analyzed the microbial activity, assessed by basal respiration and microbial biomass C and determined by substrate-induced respiration (SIR) in the different treatments. On the one hand, microbial biomass is considered to be a reliable indicator of soil fertility as it responds sensibly to changes in soil management (Powlson et al. 1987); and on the other hand, measuring respiration rates reflects the availability of C for microbial maintenance and is an indicator of the basic turnover rates in soil, thereby representing the soil microbial energy requirement at a steady-state condition (Insam et al. 1991). The metabolic quotient or specific activity of the microbial biomass (qCO_2; microbial respiration per unit biomass) was also determined, as it can be used as a measure of microbial efficiency and environmental stress (Anderson and Domsch 1993; Wardle and Ghani 1995).

Material and methods

Field trial and soil sampling

In the present study, a random design of experimental plots (four replicates, 20 m^2 per plot; each plot was 6.7 m long and 3 m wide) was set up in October, 2008 in the vicinity of Gerlos (Zillertal, Tyrol, Austria), on a ski slope in the Austrian Alps, at a height of 1,650 m above sea level (47° 14′ 40.3974″ N 12° 3′ 49.374″ E). The soil was classified as loamy–sand Semipodsol (Alois Simon, personal communication) with a pH of 5.5±0.2 and total C and N contents of 2.65 % and 0.14 %, respectively. The vegetation in the experimental area was originally forested and, according to Kilian et al. (1993), dominated by spruce (*Picea abies*). However, when the field trial was set-up, no local vegetation was found on this artificially levelled ski slope and, in turn, the area was revegetated with a commercial seed mixture "Alpin extrem" (B4) from the company Schwarzenberger (Völs, Austria), which mainly consisted of: *Poa alpina*, *Lolium perenne*, *Agrostis capillaris*, *Festuca rubra*, *F. rubra fallax*, *Festuca ovina*, *Phleum pratense*, *Poa pratensis*, *Lotus corniculatus*, *Trifolium hybridum*, and *Trifolium repens*. Both rye (*Secale cereale*) and oat (*Avena sativa*) were also added to the commercial mixture.

The cattle manure and the digestate were collected from the biogas plant located in Schlitters (Tirol, Austria). The main chemical properties of both organic amendments are shown in Table 1. Bottom wood ash was obtained from a heating and power station in Kufstein (Tirol, Austria), in which bark, sawdust, and wood chips were used as input materials. The elemental composition of the wood ash is shown in Table 2. Manure and digestate doses were chosen according to the Austrian Fertilization Guidelines (BMLFUW 2006) and the guidance of the Austrian Agriculture Ministry for the adequate use of biogas residues and anaerobically digested slurries in agriculture (BMLFUW 2007), in which doses between 70 and 100 kgN $ha^{-1}year^{-1}$ are recommended for alpine grassland, being 170 kgN $ha^{-1}year^{-1}$ the maximum permitted. On the other hand, liming is considered one of the most common and widely used treatments to overcome the problem of acidification of grassland soils in Austria (BMLFUW 2006). Specifically, doses between 0.5 and 1 CaO tha^{-1} are recommended in these areas every 4–6 years, 3 CaO $tha^{-1}year^{-1}$ being the maximum permitted (BMLFUW 2006). When the present field trial was set-up, no legislation was available in Austria regarding the use of ashes as fertilizers in grassland systems and, in turn, the above-mentioned doses recommended for lime were chosen for this study, as wood ash has properties similar to lime such as base supply and pH effect (Bougnom et al. 2010).

By combining both types of amendments (cattle manure and digestate) with three different wood ash quantities (none, 1 tha^{-1} and 3 tha^{-1}), six treatments resulted:

- D: digestate (120 kgN ha^{-1})
- DA_1: digestate (120 kgN ha^{-1})+ash (1 tha^{-1})
- DA_2: digestate (120 kgN ha^{-1})+ash (3 tha^{-1})
- M: manure (120 kgN ha^{-1})

Table 1 Main chemical properties of the cattle manure and the anaerobic digestate used in this study. Values are given for *n*=3 (standard error in brackets). All data are expressed on a dry weight basis

	Manure	Digestate
pH	8.9 (0.02)	7.6 (0.04)
EC (mS cm^{-1})	3.2 (0.05)	25.4 (1.6)
Total C (%)	39.7 (1.2)	32.5 (0.3)
Total N (%)	2.9 (0.1)	2.3 (0.03)
C/N ratio	13.5 (0.4)	14.1 (0.1)
NH_4^+ (mgkg^{-1})	2,500 (140)	15,900 (2,570)

Table 2 Elemental composition of the wood ash used in the present study. Values are given for *n*=3 (standard error in brackets). All data are expressed on a dry weight basis

Parameters	Wood ash
pH	12.81 (0.02)
EC (mS cm^{-1})	4.11 (1.49)
Total C (%)	10.6 (1.32)
N (%)	n.d.
Ca (gkg^{-1})	250
K (gkg^{-1})	26
Mg (gkg^{-1})	27
P (gkg^{-1})	7
Fe (gkg^{-1})	10
Mn (gkg^{-1})	52
S (gkg^{-1})	87
Si (gkg^{-1})	57
Cu (mgkg^{-1})	120
Zn (mgkg^{-1})	410
Cr (mgkg^{-1})	38
Ni (mgkg^{-1})	17
Pb (mgkg^{-1})	16
Cd (mgkg^{-1})	2.5
Co (mgkg^{-1})	8.4
Hg (mgkg^{-1})	0.08
Mo (mgkg^{-1})	0.63

EC electrical conductivity, *n.d.* no detectable

- MA_1: manure (120 kgN ha^{-1})+ash (1 tha^{-1})
- MA_2: manure (120 kgN ha^{-1})+ash (3 tha^{-1}).

The different treatments were spread onto the soil to an amount equivalent to 120 kgN ha^{-1} on October 15, 2008. Three soil samplings were performed on May 19, June 6, and August 25, 2009, i.e., 216, 236, and 314 days after having carried out the experimental set-up. Specifically, sampling was performed after snow melting, during the summer, and towards the end of the vegetation period, but not immediately after the fertilising period. Four composite samples were collected from each plot. Each composite sample consisting of 15 random subsamples was collected from the top 5 cm with a Pürkhauer auger (6 cm diameter). The soil samples were gently mixed and sieved (2 mm) prior to analyses.

Chemical analysis

Chemical analyses were performed after each of the three samplings. Soil samples (10 g, fresh weight) were placed into a Petri dish and oven-dried (105 °C) for at least 24 h. After this time, Petri dishes with dried soil samples were weighted and total solids were determined. The organic matter 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 contents were analyzed in dried samples using a CN analyzer (TruSpec CHN; LECO, MI, USA). EC and pH were determined in 10 g soil with 25 mL solution (deionized water/0.001 M $CaCl_2$). Inorganic nitrogen (NH_4^+ and NO_3^-) was determined in 0.0125 M $CaCl_2$ extracts, as described by Kandeler (1993a,b). Briefly, 60 mL of 0.0125 M $CaCl_2$ was added to soil samples (5 g, fresh weight), shaken for 1 h at 125 rpm and filtered. Then, 5 mL of the filtrate was mixed with 2.5 mL of a freshly prepared solution consisting of 0.3 M sodium hydroxide, 0.35 M sodium nitroprusside dihydrate and 4.02 mM sodium salicylate. One milliliter of 0.1 % (w/v) dichloroisocyanuric acid sodium salt dihydrate was added to the mix. Standard solutions containing 0.0, 1.0, 1.5, 2.0, and 2.5 μgN mL^{-1} were prepared and treated the same way as the samples. After a 30-min incubation, NH_4^+ was determined spectrophotometrically at 660 nm. Nitrate content from the soil samples was determined after adding 0.4 mL 10 % sulfuric acid to two test tubes per sample. Two copper–sulfate covered zinc granules were added to one of the tubes. Standard solutions containing 0.0, 0.5, 1.0, and 1.5 μgN mL^{-1} were prepared and treated as the samples. After an overnight incubation, NO_3^- was determined spectrophotometrically at 210 nm. Absorbance values of tubes containing zinc granules were subtracted from their corresponding granule-free tubes.

Total P (TP) and mineral P content of each sample were determined following the method proposed by Olsen and Sommers (1982). This method uses H_2SO_4 to extract P from both an ignited and unignited soil sample. Then, the organic P content was calculated as the difference between TP (ignited) and inorganic P (unignited) contents. Total nutrient and heavy metal contents were determined after moist digestion with HNO_3/HF (DIN-ISO-14869-1 2001) by inductively coupled plasma optical emission spectrometric (ICP-OES) determination (ISO-11885 2007). Mercury was determined by using atomic absorption spectrometry according to DIN EN 1483 (2007).

Microbiological parameters

Microbiological analyses were also carried out after each of the three samplings. Basal respiration was measured as CO_2 evolution from moist (60 % WHC) soil samples at 22 °C using a continuous flow infrared gas system (IRGA) (Heinemeyer et al. 1989). Readings were taken after 14 h of incubation. Microbial biomass C (C_{mic}) was determined by SIR after the addition of 1 % glucose (dry matter basis) to the samples and measuring CO_2 evolution for 8 h (Anderson and Domsch 1978), using the IRGA as above. From basal respiration and C_{mic} the metabolic quotient (qCO_2, μg CO_2–C g^{-1} C_{mic} h^{-1}) was calculated.

Plant cover

Plant cover was performed in August, as described in Kuba et al. (2008). For each plot, the total plant cover as well as soil cover by grasses and leguminous plants was estimated as a percentage of the total covered area.

Statistical analyses

Chemical and microbiological data were analyzed by repeated measures analysis of variance (ANOVAR) in which plots represented the subjects, ash concentration (0, 1, and 3 tha^{-1}), and organic amendment (manure and digestate) were fixed as the between-subject factors, and the sampling time was fixed as a within-subject factor. The differences in plant cover regarding both ash concentration and organic amendment were determined after the last sampling time using a two-way ANOVA. The normality and the variance homogeneity of the data were tested prior to ANOVA. All statistical analyses were performed with the Statistica software program v9.

Results

The chemical properties of the differently treated soils are shown in Table 3. Overall, the pH in manure-amended soils was approximately 1.2 U higher than that in digestate-treated soils (ANOVAR $F_{1,18}$=11.64, P=0.003; Table 3), irrespective of the sampling time (organic amendment×time $F_{2,36}$=1.02, P=0.37). The application of 1 t/ha of wood ash in combination with either manure or digestate resulted in similar values of soil pH to those observed in the plots in the absence of ashes (Table 3); however, adding 3 t of ashes per hectare significantly increased the levels of soil pH in both manure- and digestate-treated plots (ANOVAR $F_{2,18}$=2.75, P=0.04; Table 3). Such effects were more pronounced in May and June (ash dose×time $F_{4,36}$=5.17, P=0.002). The EC was only influenced by the sampling time (ANOVAR $F_{2,36}$=10.48, P=0.0003), with May showing the highest values (Table 3). Amending soil with digestate increased the total C content (around 1.7 times higher; Table 3), regardless of the amount of ashes (organic amendment×ash dose $F_{2,18}$=0.37, P=0.70) and the sampling time (organic amendment×time $F_{2,36}$=2.84, P=0.07). However, no significant differences were observed in the total N content among the differently treated soils (Table 3), regarding the organic amendment treatment (ANOVAR $F_{1,18}$=4.78, P=0.06) and the dose of ashes (ANOVAR $F_{2,18}$=0.47, P=0.63). Nevertheless, a decrease in this parameter was recorded over time (ANOVAR $F_{2,36}$=55.8, P=0.00001), being 2.2 and 1.5 times lower in manure- and digestate-amended soils after sampling in June and August (Table 3). Higher levels of NH_4^+ were detected in digestate-treated soils (Table 3), although this effect was dependent on the time of sampling (organic amendment×time $F_{2,36}$=3.21, P=0.04). In the digestate treatment, the content of NH_4^+ was approximately three and 1.6 times higher, relative to the manure plots, in May and June, but such differences were not found in samples collected in August (Table 3). Nitrate concentration varied with the time of sampling (Table 3), reaching the highest values in August (ANOVAR $F_{2,36}$=24.25, P=0.0001). The concentration of both NH_4^+ and NO_3^- was not significantly affected by the amount of wood ash added (Table 3), independently of whether soils were amended with manure or digestate (organic amendment×ash dose $F_{2,18}$=0.37, P=0.70 for NH_4^+ and $F_{2,18}$=1.53, P=0.75 for NO_3^-). The content of TP was influenced by the nature of the organic waste (ANOVAR $F_{1,18}$=6.37, P=0.04), with higher levels in digestate-treated plots (Table 3), regardless of the amount of ash (organic amendment×ash dose $F_{2,18}$=0.05, P=0.95) and the time of sampling (organic amendment×time $F_{2,36}$=1.95, P=0.16). However, no significant differences were found in the content of inorganic P between manure and digestate-amended soils (ANOVAR $F_{1,18}$=2.67, P=0.07; Table 3). Moreover, adding wood ash at doses of 1 or 3 t/ha resulted in levels of inorganic P similar to those observed in amended plots without ash addition (organic amendment×ash dose $F_{2,18}$=0.01, P=0.99; Table 3). Such effects related to ash addition were consistent for all the three sampling times (ash dose×time $F_{4,36}$=1.56, P=0.21; Table 3).

Table 3 Overview of the chemical properties in the differently treated soils after the sampling times in May, June, and August. Values are given for n=4 (standard error in brackets). All data are expressed on a dry weight basis

Organic waste	Ash (t/ha)	Sampling time											
		May	June	August	May	June	August	May	June	August	May	June	August
		pH			EC (μS cm^{-1})			Total C (%)			Total N (%)		
Manure	0	5.5 (0.5)	5.4 (0.4)	5.3 (0.6)	32.3 (1.9)	17.8 (8.5)	18.3 (0.6)	0.8 (0.02)	0.9 (0.02)	0.8 (0.02)	0.12 (0.01)	0.05 (0.00)	0.05 (0.00)
	1	5.9 (0.5)	5.5 (0.4)	5.5 (0.6)	26.1 (5.1)	16.0 (4.7)	16.6 (2.4)	0.9 (0.01)	1.0 (0.03)	0.9 (0.01)	0.13 (0.02)	0.07 (0.00)	0.06 (0.00)
	3	6.7 (0.4)	6.0 (0.3)	5.7 (0.3)	38.7 (3.0)	34.1 (1.9)	27.2 (8.2)	1.0 (0.03)	0.9 (0.01)	0.8 (0.02)	0.13 (0.01)	0.06 (0.00)	0.05 (0.00)
Digestate	0	4.6 (0.1)	4.7 (0.3)	4.4 (0.1)	38.1 (7.8)	12.7 (1.4)	19.6 (3.9)	1.2 (0.2)	1.4 (0.1)	1.5 (0.03)	0.13 (0.02)	0.08 (0.00)	0.09 (0.01)
	1	5.2 (0.0)	4.7 (0.1)	4.5 (0.1)	28.9 (5.1)	14.6 (1.7)	22.7 (2.8)	1.5 (0.1)	1.8 (0.09)	1.7 (0.05)	0.13 (0.02)	0.10 (0.01)	0.09 (0.01)
	3	6.0 (0.3)	5.1 (0.1)	4.6 (0.1)	36.6 (1.4)	14.7 (4.4)	15.2 (2.7)	1.1 (0.08)	1.5 (0.05)	1.1 (0.01)	0.13 (0.01)	0.08 (0.00)	0.07 (0.00)
		NH_4^+ (mg kg^{-1})			NO_3 (mg kg^{-1})			Total P (mg g^{-1})			Inorganic P (mg g^{-1})		
Manure	0	1.2 (0.2)	1.0 (0.3)	6.5 (1.1)	0.7 (0.07)	0.7 (0.05)	6.4 (1.1)	0.3 (0.01)	0.4 (0.01)	0.4 (0.03)	0.19 (0.01)	0.22 (0.01)	0.20 (0.01)
	1	1.6 (0.1)	1.0 (0.2)	3.5 (0.9)	0.7 (0.02)	0.3 (0.07)	3.1 (0.4)	0.3 (0.01)	0.4 (0.01)	0.3 (0.02)	0.20 (0.01)	0.22 (0.02)	0.18 (0.01)
	3	1.3 (0.1)	0.9 (0.1)	3.6 (0.8)	0.6 (0.01)	0.6 (0.03)	3.3 (0.2)	0.4 (0.00)	0.4 (0.01)	0.3 (0.02)	0.21 (0.01)	0.23 (0.02)	0.21 (0.01)
Digestate	0	6.3 (0.9)	1.7 (0.4)	5.4 (0.3)	3.2 (0.3)	2.9 (0.4)	4.6 (0.9)	0.4 (0.03)	0.5 (0.06)	0.5 (0.08)	0.25 (0.02)	0.35 (0.04)	0.34 (0.05)
	1	3.4 (0.1)	1.9 (0.4)	6.3 (1.1)	1.6 (0.6)	1.6 (0.1)	5.7 (0.8)	0.5 (0.08)	0.4 (0.03)	0.4 (0.05)	0.24 (0.03)	0.36 (0.05)	0.30 (0.04)
	3	3.0 (0.7)	1.0 (0.2)	4.6 (0.7)	2.0 (0.6)	1.2 (0.04)	3.0 (0.7)	0.4 (0.03)	0.5 (0.06)	0.5 (0.05)	0.26 (0.03)	0.37 (0.04)	0.34 (0.03)

EC electrical conductivity

Microbial biomass, basal respiration, and metabolic quotient data are compiled in Table 4. Similar values of microbial biomass C were found in the differently treated plots (Table 4), irrespective of amending soil with digestate or manure (ANOVAR $F_{1,18}$=0.83, P=0.38). No significant differences were found in this parameter when ashes were combined with digestate at the dose of 1 or 3 tha^{-1} (ANOVAR $F_{2,18}$=0.59, P=0.57; Table 4), and such values were similar to those observed in digestate plots without ashes (106±25 mg C kg^{-1} soil; Table 4), regardless of the sampling time (ash dose×time $F_{4,36}$=0.50, P=0.62). Soil microbial activity, assessed by basal respiration, was also not influenced either by the nature of the organic waste (ANOVAR $F_{1,18}$=1.34, P=0.27; Table 4) nor the amount of wood ash added (ANOVAR $F_{2,18}$=0.80, P=0.47, Table 4). More specifically, adding 1 and 3 t of ashes per hectare gave average values of 1.4±0.1 and 1.2±0.2 mg CO_2–C kg^{-1} soil h^{-1} in digestate-treated plots, respectively (Table 4); they were close to those reported without the addition of ash (1.1±0.1 mg CO_2–C kg^{-1} soil h^{-1}; Table 4), irrespective of the sampling time (ash dose×time $F_{4,36}$= 0.37, P=0.70). However, the digestate-amended soils had a metabolic quotient value lower than those treated with manure (ANOVAR, $F_{1,18}$=10.54, P=0.005; Table 4), and this effect did not vary with the amount of ash (organic amendment×ash dose $F_{2,18}$=0.96, P=0.40) and the sampling time (organic amendment×time $F_{2,36}$=0.48, P= 0.50). Indeed, in manure-treated plots, qCO_2 reached an average value of 15.5 and 13.2 mg CO_2–C g^{-1} C_{mic} h^{-1} after the addition of 1 and 3 t of ash per hectare, respectively (Table 4), whereas in digestate plots, qCO_2 had values of 10.9 and 11.7 mg CO_2–C g^{-1} C_{mic} h^{-1} for the low and high doses of ashes (Table 4), irrespective of the time of sampling (ash dose×time $F_{4,36}$=0.33, P=0.72).

The percentage of total plant cover was higher in those plots treated with digestate (Fig. 1), irrespective of the amount of ash (organic amendment×ash dose $F_{2,18}$=0.68, P=0.52). Specifically, adding 1 and 3 t of ash per hectare in digestate-amended soils gave a percentage of 25.25±3.4 % and 23.75±3.5 %, respectively (Fig. 1). These values were not significantly different from those found in the absence of ashes (19.75±2.8 %; ANOVA $F_{2,18}$=0.06, P=0.94). In the manure treatment, values of 15.25±2.1 % and 14.25±2.8 % were recorded for total plant cover when wood ash was applied at doses of 1 and 3 t/ha (Fig. 1). In addition, digestate-treated plots showed a higher proportion of leguminous plants than those amended with manure (ANOVA $F_{1,18}$=30.80, P=0.00003, Fig. 1). As occurred with the total plant cover, such differences between manure- and digestate-amended soils were not dependent on the amount of ash (organic amendment×ash dose $F_{2,18}$=0.63, P=0.54). The percentage of leguminous plants cover was about 11.5± 1.5 % after the addition of ashes in soils treated with digestate (Fig. 1), whereas a value close to 5 % was reported for leguminous proportion in manure plots amended with either 1 or 3 t of ashes per hectare (Fig. 1). Nevertheless, the fraction of grasses was not significantly affected by the nature of the organic waste (ANOVA $F_{1,18}$=0.62, P=0.44) and the amount of ash (ANOVA $F_{2,18}$=0.13, P=0.87) (Fig. 1).

Discussion

To date, most of the previous studies have primarily been focused on evaluating separately the effects of wood ash and digestate on soil properties (Saarsalmi et al. 2001, 2004; Augusto et al. 2008; Perucci et al. 2008; Ferreiro et al. 2011; Goberna et al. 2011; Walsh et al. 2012), and, in turn, little is yet known about how the combined use of both products influence on the plant–soil system (Bougnom et al. 2012). In this sense, our study shows that the wood ash generated as a

Table 4 Overview of the microbiological properties in the differently treated soils after the sampling times in May, June, and August. Values are given for n=4 (standard error in brackets). All data are expressed on a dry weight basis

		C_{mic} (mg C kg^{-1} soil)			Basal respiration (mg CO_2–C kg^{-1} soil h^{-1})			qCO_2 (mg CO_2–C g^{-1} C_{mic} h^{-1})		
		Sampling time								
Organic waste	Ash (t/ha)	May	June	August	May	June	August	May	June	August
Manure	0	85 (10)	80 (10)	107 (24)	0.9 (0.03)	1.4 (0.4)	1.6 (0.4)	11.5 (1.7)	17.1 (1.6)	14.9 (1.6)
	1	107 (27)	98 (18)	104 (3)	1.8 (0.6)	1.7 (0.5)	1.4 (0.1)	16.4 (2.8)	16.9 (1.7)	13.1 (0.9)
	3	162 (33)	105 (17)	94 (14)	2.2 (0.3)	1.7 (0.4)	0.9 (0.2)	14.3 (1.1)	15.6 (1.2)	9.8 (1.3)
Digestate	0	107 (12)	106 (26)	105 (17)	1.0 (0.2)	1.0 (0.1)	1.0 (0.2)	8.8 (0.5)	10.7 (2.0)	9.5 (1.2)
	1	132 (34)	155 (14)	95 (12)	1.6 (0.4)	1.4 (0.4)	1.1 (0.1)	12.2 (1.4)	8.9 (0.6)	11.7 (1.0)
	3	121 (5)	97 (6)	88 (11)	1.3 (0.1)	1.1 (0.2)	1.1 (0.2)	10.5 (0.8)	11.6 (1.2)	12.0 (1.7)

C_{mic} microbial biomass carbon

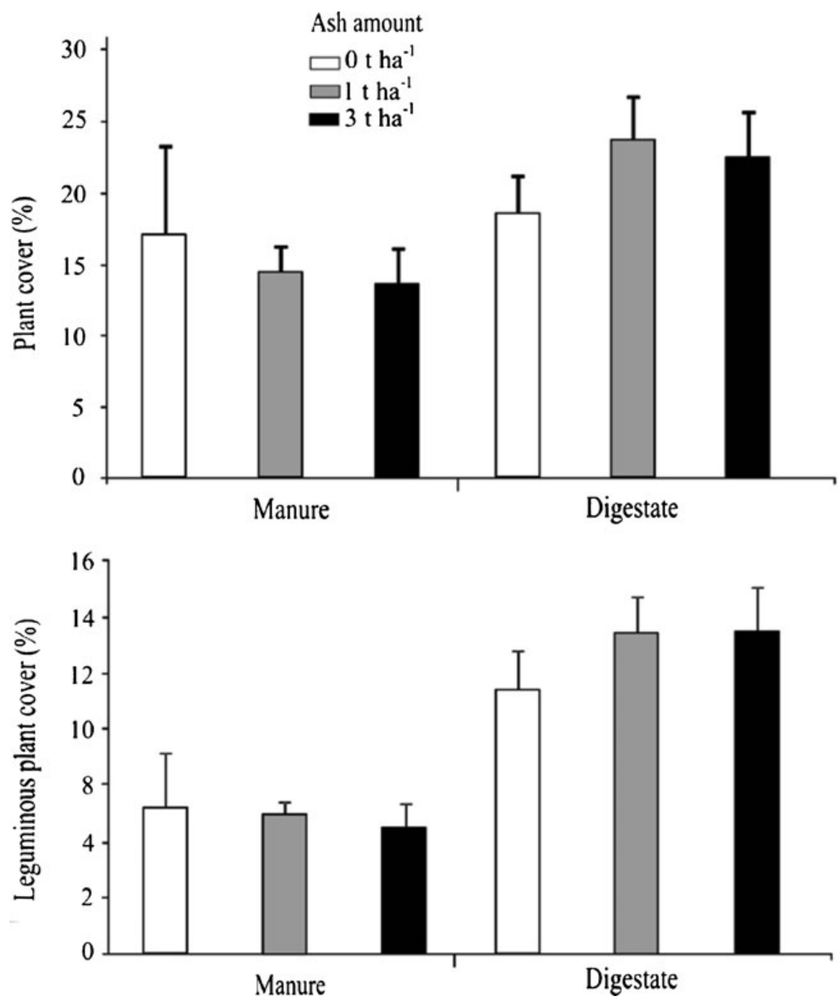

Fig. 1 Percentage of the total plant cover and the fraction of leguminous in the differently treated soils after the sampling time of August. Values are means ± SE

by-product of combustion, whether for heat or power generation, has a potential use as a fertilizer in grassland systems as, in general, no harmful effects were observed in soil properties from chemical and microbiological viewpoints, even when ashes were combined with anaerobic digestate at the dose of 3 tha^{-1}. These findings offer the possibility to promote the sustainable use of both wood ash and digestate as soil fertilizers and to find a suitable recycling strategy for their disposal. Indeed, in a recent work by Goberna et al. (2011) highlighted that more experimental data needs to be gathered in order to better define the benefits and risks of land-spreading biogas digestates, thereby replacing the use of fresh manure as a fertilizer.

In the present study, amending soil with manure led to a larger increase in soil pH regardless of the sampling time in comparison with the digestate, probably because a lower pH was found in this latter product (Table 1). Since no additional amendments were incorporated over time in order to compensate the system consumption, lower pH values were recorded in the differently treated soils at the end of the experiment, reaching a value close to the initial soil pH (5.5±0.2). On the other hand, the presence of quickly soluble compounds such as oxides, hydroxides, and carbonates could be responsible for the rapid increase in soil pH following wood ash addition (Ozolinčius et al. 2007). Indeed, in a previous work, these authors found that pH may rise as much as 2.7 U in the O horizon 3 months after the application of 5 tha^{-1} of raw wood ash (Ozolinčius et al. 2005). The increase in pH with ash amendment has been shown to be more pronounced for soils with a low pH and low organic matter content (Ohno 1992; Perucci et al. 2006), as analyzed in the present study. Additionally, in this study, the pH rise was found to be proportional to the amount of wood ash added in those plots amended with either digestate or manure, confirming other works investigating the potentiality of using ashes as soil fertilizers (Perucci et al. 2008) or as compost additives (Kuba et al. 2008; Bougnom et al. 2009). In contrast to pH, no significant differences were observed in the EC between ash-treated soils and those without ashes, irrespective of whether soils were amended with manure or digestate. 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 1 or 3 t of ashes per hectare.

The fact that both organic amendments were applied at the same N rate could explain why no significant differences were recorded in the amount of total N between manure- and digestate-treated soils. For N-deficient soils, as those being analyzed in this study, it is common practice to use this type of N-containing fertilizers; hence, we considered not to include wood ash alone as a treatment in the experimental set-up, as it contains a very small amount of N due to its volatilization during combustion (Knapp and Insam 2011). This fact could explain why no significant effects were found in soil N status over time regardless of the dose of application of ashes. A similar trend was observed regarding the impact of ashes in the C content of the differently treated soils, as this element is mostly oxidized during combustion. It is widely known that during the process of anaerobic digestion, the organic N compounds are mineralized, resulting in increased levels of the soluble forms of inorganic N, mainly ammonia (Möller and Stinner 2009). Accordingly, a higher NH_4^+ content was found in the initial digestate compared to the untreated manure (Table 1), thereby leading to levels of NH_4^+ in digestate-amended soils significantly higher than those in manure-amended soils. However, no significant differences were detected in the differently treated plots regarding NO_3^- content. Alburquerque et al. (2011) reported that the changes in NH_4^+ and NO_3^- concentrations in digestate-amended soils are clearly influenced by the degree of stability of the added material. They found that amending soil with digestates that have a high proportion of labile organic matter led to a reduction in the content of both mineral N forms in the first weeks of the incubation period, which may be accompanied by the immobilization of inorganic N in soil as a consequence of the intense microbial activity, along with a lack of oxygen in the soil leading to denitrification.

Furthermore, the digestate-amended soils had a higher content of TP relative to those treated with manure irrespective of the dose of ashes, which points to the enrichment of mineral fractions of P during digestion. The higher content of P in digestate-amended soils could have contributed to the increase in the percentage of leguminous cover in comparison with that in manure-treated soils, even for the wood ash treatment at 3 tha^{-1}. In accordance to this, Canellas et al. (2004) found that tropical soils covered with leguminous plants have higher labile organic P than those from secondary tropical forest and grass areas. It must be also mentioned that increasing soil P levels affects positively the yield of both mycorrhizal and nonmycorrhizal soybeans (Asimi et al. 1980).

The dissolution of wood ash in soil and the rate at which nutrients become plant available following ash addition depends greatly on several factors including the initial composition of the ash, along with the shifts in pH-dependent soil chemical equilibria and the changes in the level of activity of soil microbial communities (Demeyer et al. 2001). In the present study, amending soil with ashes did not affect the content of total and inorganic P in either manure- or digestate plots, even when ashes were applied at the dose of 3 tha^{-1}. This could be due to the low availability of P in the ash, as previously shown by Khanna et al. (1994). According to dissolution tests based on different ash/water ratios, these authors observed that K, Ca, and Mg dissolved very quickly, whereas they detected that P remained relatively insoluble. Moreover, as mentioned above, an increase in soil pH was observed following ash addition, which could have also influenced the solubility and availability of P in soil (Demetz and Insam 1999; Schiemenz et al. 2011). Additionally, in acid soils, phosphate ions tend to precipitate with Fe and Al, thereby forming insoluble compounds and reducing the content of H_2PO_4 taken up by the plants (Omil et al. 2011).

A principal requirement for an organic amendment for its safe use in soil is its degree of stability (Insam and de Bertoldi 2007). Biological parameters such as respiration rates (CO_2 evolution rate and/or O_2 uptake rate) and microbial biomass measurements have been proposed for evaluating the stability of the added material (Bernal et al. 2009); such parameters have also been shown to provide rapid information on soil quality because of their sensitivity to change (Nannipieri et al. 1990, 2003). In general, the response of soil microbial properties to organic and conventional amendments is often studied in the long-term (Ferreras et al. 2006; Monaco et al. 2008; Walsh et al. 2012), although Gil-Sotres et al. (2005) and Dinesh et al. (2010) have pointed out the possibility of using these properties to assess the short-term impact of agricultural management. Thus, it should be noted that in the present study, we necessarily 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 wood ash impact in soil in combination with anaerobic digestates. Specifically, we did not find significant differences regarding the microbial biomass and activity between soils amended with the untreated manure and those with the digestate, irrespective of the sampling time. The impact of the digestate on soil is expected to be largely dependent on its quality, which is related to the efficiency with which the process of anaerobic digestion took place. In line with this, Odlare et al. (2011) established that the C in biogas residues is, in fact, more easily degradable than that of composts mainly due to the fact that the mineralization is less efficient under anaerobic conditions. This could have minimized the differences regarding the impact of both manure and digestate in soil microbial biomass and its activity. The ability of the native soil microbiota to outcompete the allochthonous microorganisms applied with the amendments may have also contributed to the lack of differences between the amended soils. This fact has been previously shown for compost amendments concerning fungal denaturing gradient gel electrophoresis (DGGE) profiles (Ros et al. 2006a,b). These authors found that the date of sampling contributed more to modifications in the soil fungal community than treatment effects. Furthermore, our study is in accordance with previous works showing no effects on soil microbial biomass and activity after adding wood ash in combination with organic wastes (Fritze et al. 1994; Bougnom et al. 2012). The use of ash could have contributed to the absence of detrimental effects on the soil microbiota, as this type of ash is usually characterized by low content in heavy metals and xenobiotic contaminations (Stockinger et al. 2006; Knapp and Insam 2011). However, Jokinen et al. (2006) observed an increase in microbial activity (thymidine incorporation) due to ash amendment, along with a shift in the microbial community composition, assessed by PCR-DGGE profiles. Moreover, Mahmood et al. (2003) reported increases in the fungi/bacteria ratio that were related to increases in wood ash addition. Contrarily, other authors detected a reduction in the soil respiration, microbial biomass, and enzymatic activities following the application of fly ash (Sharma and Kalra 2006), and such effects were proportional to the amount of ash. Overall, such discrepancies among the aforementioned studies regarding ash treatment may be due to differences in soil properties, pretreatment and dose of application of the ashes, length of the experiment, and parameters chosen for analysis, among others.

Despite the lack of differences in microbial biomass and activity among the differently treated plots, amending soil with anaerobic digestate led to lower values of qCO_2 relative to the untreated manure. Interestingly, combining the digestate with either 1 or 3 t of ashes per hectare resulted in similar values to those observed in the treatment without ashes, which reinforces the harmless action of ashes on the soil microbiota (Bougnom et al. 2012). This fact is of utmost importance regarding the mineralization of organic matter in ash-treated soils, as during decomposition all organic matter goes through the microbial decomposer pool. In the case of Insam et al (2009), the combination of wood ah and digestate led to a change in the composition of microbial communities assessed by PCR-DGGE profiles and microarray analyses, compared to control and digestate alone; however, the metabolic quotient remained unaffected among the treatments. Thus, it should be noted that this index has been also criticized by Wardle and Ghani (1995), as these authors found that it can be insensitive to disturbance and ecosystem development, thereby confounding the effects of disturbance with those of stress. Additionally, the significance of the increase of qCO_2 in distorted ecosystems is unclear because it may be attributed to a reduction in the substrate utilization efficiency by the microbiota, to the predominance of zymogene flora (r-strategists) over the autochthonous flora (K-strategists), as well as to the alteration of the bacteria/fungi ratio, since they have different carbon use strategies (Dilly and Munch 1998).

Conclusions

The combined use of wood ash with enriched N organic wastes can primarily constitute an efficient way for the disposal and recycling of both products and additionally, it may constitute an environmentally friendly alternative to mineral fertilizers for acid soils. On the one hand, our findings revealed that amending soils with digestate resulted in a higher nutrient content (NH_4^+ and TP), as well as in a more efficient soil microbial community relative to those plots treated with manure. Such effects were accompanied by a higher percentage of plant cover, particularly of leguminous plants. Interestingly, on the other hand, these patterns were maintained in the digestate-amended plots even when the ash was added at a rate of 3 tha^{-1}, indicating its harmless action on soil properties and ultimately, suggesting its use as a valuable additive in grassland systems. Nevertheless, no specific beneficial effects of ash have been found in this experiment, thereby leading to the interest for further trials to evaluate the long-term effects of ashes in grassland soils and to determine the maximum rate at which they can be applied without having a negative impact on soil properties. Ash addition should, thus, be considered primarily for soils that have a clear lime requirement or are deficient in any of the micronutrients contained in ash.

Acknowledgments This research was supported by the K-Regio Center BioTreaT (Biological Treatment and Recycling Technologies). Marina Fernández-Delgado Juárez is in receipt of a PhD fellowship from Nachwuchsförderung from the University of Innsbruck. María Gómez Brandón is financially supported by a postdoctoral research grant from Fundación Alfonso Martín Escudero. The authors thank Skiliftzentrum Gerlos for providing a trial area and the Österreichische Bundesforste A.G. for their kind cooperation. They also acknowledge Paul Fraiz for his highly valuable help in language editing.

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