Environmental Pollution 224 (2017) 581–589

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Wood ash application increases pH but does not harm the soil mesofauna☆

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ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form
13 February 2017
Accepted 18 February 2017
Available online 27 February 2017

Keywords:
Wood ash
Reproduction
Folsomia candida
Onychiurus yodai
Hypoaspis aculeifer
Enchytraeus crypticus
Soil pH
Osmolality

ABSTRACT

Application of bioash from biofuel combustion to soil supports nutrient recycling, but may have unwanted and detrimental ecotoxicological side-effects, as the ash is a complex mixture of compounds that could affect soil invertebrates directly or through changes in their food or habitat conditions. To examine this, we performed laboratory toxicity studies of the effects of wood-ash added to an agricultural soil and the organic horizon of a coniferous plantation soil with the detrivore soil collembolans *Folsomia candida* and *Onychiurus yodai*, the gamasid predaceous mite *Hypoaspis aculeifer,* and the enchytraeid worm *Enchytraeus crypticus*. We used ash concentrations spanning 0–75 g kg^{-1} soil. As ash increases pH we compared bioash effects with effects of calcium hydroxide, $Ca(OH)_2$, the main liming component of ash. Only high ash concentrations above 15 g kg^{-1} agricultural soil or 17 t ha^{-1} had significant effects on the collembolans. The wood ash neither affected *H. aculeifer* nor *E. crypticus*. The estimated osmolalities of $Ca(OH)_2$ and the wood ash were similar at the LC_{50} concentration level. We conclude that short-term chronic effects of wood ash differ among different soil types, and osmotic stress is the likely cause of effects while high pH and heavy metals is of minor importance.

1. Introduction

Wood ash from combustion of various types of wood in power plants is currently regarded as a waste product to be recirculated to plantations and cultivated fields in small amounts (Miljøstyrelsen, 2008a). Environmental effects of ash application due to its complex mixture of beneficial and detrimental compounds have been observed in several studies (e.g. Augusto et al., 2008; Nabeela et al., 2015; Nieminen, 2011). Burning of mixed organic fuels such as straw, woodchips, green waste and logs, produces a mixed quality ash of variable chemical content (Pitman, 2006). It contains a mixture of salts, mostly with cations of Ca, K, Fe, Al, Mn, Na, Mg and various trace elements in lesser amounts (Ozcan et al., 2013). Due to its high content of oxides and hydroxides, the wood ash has alkaline properties and is frequently used as a pH raising agent in acidic soils (Heviánková et al., 2014; Neina and Dowuona, 2014; Ohno and Susan Erich, 1990; Ozcan et al., 2013). Because of the liming effect we hypothesize that the soil pH increase from ash application could be a main contributor to toxicity, as it is know that pH above 7 inhibits collembolan reproduction (Greenslade and Vaughan, 2003; Jänsch et al., 2005). Otherwise, osmotic stress from soluble salts and heavy metal are among candidates explaining responses to bioash by soil invertebrates.

Bioash affects soil invertebrates and their food and habitat conditions (Haimi et al., 2000; Liiri et al., 2002; Nieminen et al., 2012). Mesofauna, i.e. invertebrates 0.2–2 mm in size, plays an important role in the decomposition of soil organic matter, not only by consuming litter, but also by regulating the microbial community. Thereby, and via direct predation on other faunal groups, they are essential components of the soil food web (Sackett et al., 2010). The collembolans *Folsomia candida* (OECD, 2009) and *Onychiurus yodai*, the mite *Hypoaspis aculeifer* (OECD, 2008) and the

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http://dx.doi.org/10.1016/j.envpol.2017.02.041
0269-7491/

enchytraeid-worm *Enchytraeus crypticus* (ISO, 2004; OECD, 2004) are soil mesofauna model organisms that are widely used to uncover ecotoxicological effects of xenobiotics (Arp et al., 2014; Ke et al., 2004; Larsen et al., 2008; Nakamori et al., 2008; Sverdrup et al., 2007). The four species represent dominant types of mesofauna.

Apart from the composition of ash, the soil type and application rate are important factors determining how the ash affect the soil biota (Pitman, 2006). Hence, we used the four species *F. candida*, *O. yodai*, *H. aculeifer* and *E. crypticus* as indicators to test the ecotoxicological effects of ash in two types of soil either from an agricultural field or from a spruce plantation. Therefore, we will assess the effects of ashes on the population performance of these four species in two types of soil and in addition assess effects of an increase in pH through liming. Hence, our study addresses the following hypotheses: Wood ash could potentially affect soil mesofauna, and the species *F. candida*, *O. yodai*, *H. aculeifer* and *E. crypticus* could respond differently; effects of the wood ash could differ depending on the soil type, and factors involved could be liming and osmotic changes. This is essential knowledge needed to improve the management of ashes and thus minimize damage to the soil ecosystem.

2. Methods

2.1. Test substances and test soil

We used a mixed bottom and fly ash made from burning mixed wood chips at a district heating plant located in Galten, Denmark. For comparison an additional type of wood bottom and fly ash from burning *Picea abies* wood chips at a district heating plant located in Brande, Denmark, was employed to test if similar types of ashes elicited the same ecotoxicological response. Prior to use, ash samples were crushed and subsamples were chemically analysed (Hovmand et al., 2008; Miljøstyrelsen, 2008b). The concentrations of nitrogen (N) and carbon (C) were determined on 0.5 g ash subsamples by infrared absorption spectroscopy (IR) after dry combustion in an oven (LECO-CNS 2000). From each sample, 150 mg was digested in nitric acid (HNO_3) in Polytetrafluoroethylene (PTFE) bombs in a microwave oven (CEM, DMS-2000). After digestion the concentration of P, K, Ca, Mg, Mn, Zn, Cu, As, Cd, Pb, Cr, and Ni was measured by inductively coupled plasma atomic emission spectroscopy ICP-AES (Perkin Elmer, optima 3000 XL) (Hovmand et al., 2008; Miljøstyrelsen, 2008b). All concentrations of solid material refer to dry weight at 55 °C (Table 1).

A loamy sand was sampled from the ploughing layer of an agricultural field in Foulum, Jutland, Denmark, N 56° 29.699′, E 9° 33.601′ with soil characteristics reproduced from Sørensen et al. (2003) in Table 2. A spruce forest soil was sampled from the O-horizon of Gedhus coniferous plantation (Jutland, Denmark, N 56° 16.566′, E 9° 6.400′) and consisted of decaying plant material without mineral particles (Table 2). Cation exchange capacity (CEC) was measured according to Stuanes et al. (1984) and C and N were measured with a FLASH 2000 EA NC Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Both types of soil were defaunated by heating at 80 °C for 24 h and sieved through 2 mm mesh prior to use (e.g. Scott-Fordsmand et al., 1997; Sverdrup et al., 2001).

2.2. Test species

F. candida, the Berlin strain, and *E. crypticus,* the Huckingen strain, GenBank accession no. GU902055.1, were cultured at Aarhus University (Silkeborg, Denmark) at 20 ± 1 °C with a 12:12 h dark: light cycle. *O. yodai*, GenBank accession no. KF311741.1, was originally from Institution of Soil Science, Chinese Academy of Science (Nanjing, China). *H. aculeifer*, GenBank accession no. FM210170.1 was obtained from a commercial supplier (EWH Bio-Production Aps, Tappernøje, Denmark). *E. crypticus* were fed oatmeal (Quaker Oats Company), while *F. candida* and *O. yodai* were fed Baker's yeast (De Danske Gærfabrikker A/S, Grenå, Denmark).

2.3. Toxicity test

The ecotoxicological tests were performed according to the principles of OECD guidelines (OECD, 2004, 2008, 2009) and the details are listed in Table 3. The Galten ash was mixed with the soil to obtain increasing nominal concentrations (Table 3), selected according to the results of range-finding tests (Supplementary material). For *F. candida*, *O. yodai* and *H. aculeifer*, 30 g loamy sand and 20 g moist Gedhus soil were put into each container, respectively. For *E. crypticus,* 20 g loamy sand were added into each container, and for Gedhus soil five gram moist soil were used in each container. The water content was adjusted to fifty percent of the water holding capacity (WHC) of the mixture of soil and wood ash.

Ten individuals of *F. candida* at the age of 9–12 days (d) were transferred by a pipette tip connected to a low pressure suction system into each testing unit. A test unit consisted of a plastic cylinder, 5.5 cm high and with an inner diameter of 6.0 cm, which was closed at the top and bottom with lids during exposure. The test included eight replicates for the control and four replicates for each treatment and lasted 28 d. Five males and ten females of *O. yodai* at the age of 24–27 d were exposed in soil contaminated with ash for 35 d. Eight replicates were made for control and two replicates were made for each treatment. After addition of the collembolans, approximately 30 mg of baker's yeast was added on

Table 1
Content of plant nutrients and heavy metals in the ashes made from combustion of energy wood and soil used in the ecotoxicological tests. CV%: Coefficient of Variability.

	Macronutrients					Micronutrients		Heavy metals				
	g kg^{-1}					mg kg^{-1}		mg kg^{-1}				
	Ca	K	Mg	Mn	P	Cu	Zn	As	Cd	Cr	Ni	Pb
Galten Ash	**116**	**33**	**14**	**11**	**17**	**103**	**572**	**4.4**	**2.2**	**28**	**27**	**17**
CV %	15	11	10	14	11	17	2	17	48	11	10	32
Brande Ash	**128**	**29**	**12**	**8**	**10**	**63**	**331**	**1.3**	**3.9**	**13**	**22**	**10**
CV %	14	9	21	15	28	13	12	11	8	7	22	11
Foulum soil 0–10 cm	**3.6**	**1.4**	**1.3**	**0.5**	**1.3**	**31**	**27**	**5.6**	**0.45**	**13**	**4.4**	**14**
CV %	11	6	7	5	6	5	7	5	2	9	28	8
Gedhus soil O-horizon	**0.9**	**0.5**	**0.8**	**20**	**0.8**	**7**	**35**	**2.7**	**0.64**	**3.9**	**3.3**	**59**
CV %	62	7	32	19	4	20	26	41	30	10	16	28

Table 2
Physicochemical properties of the Foulum agricultural soil and the Gedhus spruce forest O-horizon.

Origin of Soil	pH (H_2O)	Bulk density	Water holding capacity	Organic matter	Total carbon	Total nitrogen	Cation exchange capacity	Clay	Silt	Sand
		g cm^{-3}	g g^{-1}	g kg-1	g kg^{-1}	g kg^{-1}	cmol(+) kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Foulum soil	6.3	1.16	0.46	35	21	2.1	–	80	125	760
Gedhus soil	3.4	0.18	2.60	≈100%	444	15	12.9	0	0	0

Table 3
Basic experimental conditions of the single species concentration-response tests in Foulum soil and Gedhus soil.

Test species		At test start		Test duration Days	Food resources	Origin of soil	Soil amount g	Replication	Nominal concentration series g ash kg^{-1} soil
		Age Days	Number of individuals						
Toxicity test									
Galten ash	*Folsomia candida*	9–12	10	28	Baker's yeast	Foulum Gedhus	30 20	Control: 8 Treatment: 4	0, 5, 10, 12, 15, 17, 20 0, 5, 10, 15, 20
Galten ash	*Onychiurus yodai*	24–27	15	42	Baker's yeast	Foulum Gedhus	30 20	Control: 8 Treatment: 2	0, 5, 10, 15,20, 25, 30, 35, 40, 50, 60, 75
Galten ash	*Hypoaspis aculeifer*	32-35 d Female	10	21	*F. candida* juveniles	Foulum Gedhus	30 20	4	0, 50
Galten ash	*Enchytraeus crypticus*	Female, adult	10	21	Oatmeal	Foulum Gedhus	20 5	4	0, 50
Liming test									
Galten ash	*Onychiurus yodai*	Adult	10	14	Baker's yeast	Foulum	30	2	0, 20, 30, 40, 50, 75
Brande ash	*Onychiurus yodai*	Adult	10	14	Baker's yeast	Foulum	30	4	0, 20, 30, 40, 50, 75
$Ca(OH)_2$	*Onychiurus yodai*	Adult	10	14	Baker's yeast	Foulum	30	4	0, 1, 3, 5, 10, 15

the surface of soil.

Only one concentration, i.e. a limit test *sensu* OECD (2008), of 50 g Galten ash kg^{-1} dry weight soil was applied to soil in the tests with *H. aculeifer* and *E. crypticus*, because there were no significant differences observed in the range-finding test for both soil types (Supplementary material). Thus, four replicates were made for control and treatment. Ten female *H. aculeifer* at the age of 35–38 days were added into each container, same with containers used for collembolan. More than 300 *F. candida* juveniles were added to each container to ensure sufficient food supply for *H. aculeifer*. Ten adult *E. crypticus* were exposed to contaminated soil in each container, which was plastic cylinder (5 cm high, inner diameter 3.5 cm) closed at the top and bottom with lids during exposure (upper lid was perforated). After addition of animals, approximately 30 mg of oatmeal was added to the surface of the soil as the food of *E. crypticus*. The oatmeal was boiled, dried, and crushed prior to use to avoid excessive growth of fungi in the test containers. The testing periods for *H. aculeifer* and *E. crypticus* were both 21 d.

The tests were conducted at 20 ± 1 °C with a 12:12 h dark: light cycle. After exposure, *E. crypticus* test soil was put into the sieve filled of water and heated with light from above and collected from the bottom of the sieve, while *F. candida*, *O. yodai* and *H. aculeifer* were collected with a controlled temperature gradient extractor (Macfadyen, 1961; Petersen, 1978). All animals were counted manually under the microscope.

2.4. Liming with $Ca(OH)_2$

In order to isolate the effect of high pH from other chemical effects of the ash constituents, we performed additional *O. yodai* survival tests with three treatment concentration series of $Ca(OH)_2$ liming, Galten ash and Brande ash in the loamy sand. *O. yodai* was selected because it was the only species where the survival was sensitive to the ash. The nominal concentrations were listed in Table 3. *O. yodai* was exposed for 14 d under the same conditions as in the toxicity test, except that the soil spiked with $Ca(OH)_2$ was incubated for one month before test start. The purpose of this long pre-incubation was to stabilize the pH of the loamy sand, because the pH was observed to decrease dramatically within the first month after spiking to soil (data not shown). After exposure, *O. yodai* was extracted and counted in the same way as done for the other collembolans.

2.5. pH measurement

pH_{H_2O} was measured according to the OECD guidelines (e.g. OECD, 2009). For every ash treatment concentration one additional container was incubated and used for pH measurement in two subsamples of 5 g soil in 25 mL distilled water. pH measurements were made every two weeks from the beginning to the end of each test, i.e. three times.

2.6. Conversion of nominal concentration from laboratory into field scale

In order to compare the results with reported field study results, we converted the nominal concentration for our laboratory test to field scale according to the equation:

$$C = \rho \cdot d \cdot c$$

Where C is the concentration of ash calculated by area, t ha^{-1},

ρ is the bulk density of soil, g cm^{-3},
d is the distance of ash seeping into soil, dm, and
c is the nominal concentration in soil, g kg^{-1}

The bulk density of the loamy sand and the Gedhus soil are 1.16

(Jensen et al., 1999) and 0.18 kg L^{-1}, respectively. In the loamy sand, we assumed that the wood ash would be evenly distributed in the top 10 cm of the ploughing layer. In the Gedhus soil, measurements of pH changes after ash application suggest that the wood ash was distributed only in the upper 2 cm of the profile (data not shown). Thus, if the nominal concentration is 1 g kg^{-1}, the concentration of ash calculated by area will be 1.16 t ha^{-1} in the loamy sand and 0.09 t ha^{-1} in Gedhus soil.

2.7. Statistical analysis

R version 3.1.2 (R Core Team, 2014) was used for statistical analyses. We used one-way ANOVA to assess significant differences between treatment and control of normally distributed reproduction data and binomially distributed survival data. Non-parametric Kruskal–Wallis test was employed when data remained non-binomially and non-normally distributed even after log transformation. To examine the interaction between soil type and wood ash treatments, we conducted a two-way ANOVA for reproduction of *F. candida* and *O. yodai*. The survival of *F. candida* and *O. yodai* was tested with Scheirer-Ray-Hare extension of the Kruskal-Wallis Test (Sokal and Rohlf, 2012), as it could not be fitted with a binomial generalized linear model. The R procedure of Scheirer-Ray-Hare test was employed according to the online tutorial for book Research Methods for the Biosciences (Holmes et al., 2016a, 2016b). No observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were determined by comparing the treatments and control using Dunnett's test with the *multcomp* package (Hothorn et al., 2008, 2016). Results with significant effects with increasing concentrations of ash were fitted to a log-logistic dose response model employing the *drc* package (Ritz and Streibig, 2005):

$$y = c + \frac{d - c}{1 + e^{b(\ln(x) - \ln(EC_{50}))}}$$

Where y is the result of reproduction or survival, x is the nominal ash concentration in g kg^{-1} dry soil, b is the slope, c is the lower limit of the response, d is the upper limit of the response (Ritz and Streibig, 2005). The effective dose was estimated by the function ED of the *drc* package (Ritz and Strebig, 2015).

Student's t-test was used to compare the different effects of ash on *E. crypticus* and *H. aculeifer* between control and treatment.

3. Results

3.1. F. candida

Ash amendment in the loamy sand resulted in significantly different reproduction between treatments and control (one-way ANOVA, $F_{6, 25} = 45.1$, $p < 0.05$) (Fig. 1). The NOEC value was 17 g kg^{-1} and the LOEC value was 20 g kg^{-1} (Table 4). Adding ash to the loamy sand inhibited the reproduction of *F. candida* resulting in an EC_{50} value of 19.5 [-3.82–42.8] g kg^{-1}. No effects were observed on the survival in any of the two soil types within the range of the tested concentrations. Neither were there any negative effects on the number of *F. candida* juveniles in Gedhus soil at the tested concentrations (Fig. 1). However, the range-finding test indicated that stimulation could occur (see Supplementary material). The two-way ANOVA and Scheirer-Ray-Hare Test showed that F. candida survival and reproduction was significantly lower in the Gedhus soil compared to the loamy soil (see Supplementary material, Table A2).

3.2. O. yodai

A significant decrease of the reproduction (one-way ANOVA, $F_{11, 17} = 19.5$, $p < 0.05$) and the survival (Kruskal–Wallis, $p < 0.05$) of *O. yodai* started at 15 g kg^{-1} in the loamy sand (Fig. 1). According to logistic curve fitting, EC_{50} values for the wood ash effect on *O. yodai* were 8.15 [0.608–15.7] g kg^{-1} and 40.0 [36.8–43.2] g kg^{-1} for reproduction and survival respectively (Table 4). There were no significant differences observed for the survival and reproduction of *O. yodai* between treatments and control in the Gedhus soil (Fig. 1). The two-way ANOVA and Scheirer-Ray-Hare Test revealed that the *O. yodai* survival was significantly lower in Gedhus soil than in loamy soil, while the *O. yodai* reproduction didn't differ in the two types of soil (see Supplementary material, Table A2).

3.3. E. crypticus and H. aculeifer

The Galten ash had no significant effect on *H. aculeifer* and *E. crypticus* in the loamy sand, while both survival and reproduction of *H. aculeifer* and *E. crypticus* were significantly increased by ash addition in Gedhus soil ($p < 0.01$) (Fig. 2).

3.4. $Ca(OH)_2$ liming

The liming resulted in a significant decrease of the survival of *O. yodai* after exposure to $Ca(OH)_2$ (one way ANOVA, $F_{5,18} = 170$, $p < 0.05$), Galten ash (one way ANOVA, $F_{5,6} = 21.4$, $p < 0.05$) and Brande ash (one way ANOVA, $F_{5,18} = 28.2$, $p < 0.05$) in the loamy sand for 14 d (Fig. 3). The LC_{50} values are given in Table 4 in terms of ash concentrations and in terms of measured pH. The LC_{50} value of $Ca(OH)_2$ converted to osmolality was 1565 [-435, 3357] mOsm kg^{-1} (Martin et al., 2011).

4. Discussion

We observed that effects of wood ash are highly dependent on the properties of the testing substrate. Only high concentrations of wood ash (>15 g kg^{-1}/17.4 t ha^{-1}) applied to agricultural soil had significantly negative effects on collembolans but no effect on the enchytraeid and the gamasid mite. Alkaline soil conditions are harmful to collembolans (Greenslade and Vaughan, 2003; Jänsch et al., 2005), and as the wood ash increased the pH of the loamy sand from 7 to 9 (Fig. 4) it caused significant effects. The heavy metal concentrations were below the sensitivity of the test species.

4.1. H. aculeifer

The ash treatments increased the reproduction of *H. aculeifer* in Gedhus soil, while ash had no effect in the loamy sand. From the majority of the habitats of *H. aculeifer* (Murphy and Sardar, 1991), it might be concluded that it has a preference for neutral soils, with some tolerance to acid conditions as it was reported for coniferous forests (Huhta et al., 1986). Thus, the pH range (Fig. 4) obtained in our study will not cause stress to *H. aculeifer*. This explains why wood ash didn't cause significant effects on survival in both types of soil and for the reproduction in the loamy sand. The reproduction of *H. aculeifer* in control soil is lower than in the ash amended Gedhus soil, which could be explained by inhibition of the fecundity and that cuticles of juvenile *H. aculeifer* is less acid protective than the scleroticized cuticle of adults.

4.2. E. crypticus

The ash in Gedhus soil had a positive effect on survival in the definitive test. So it increased the survival of enchytraeids

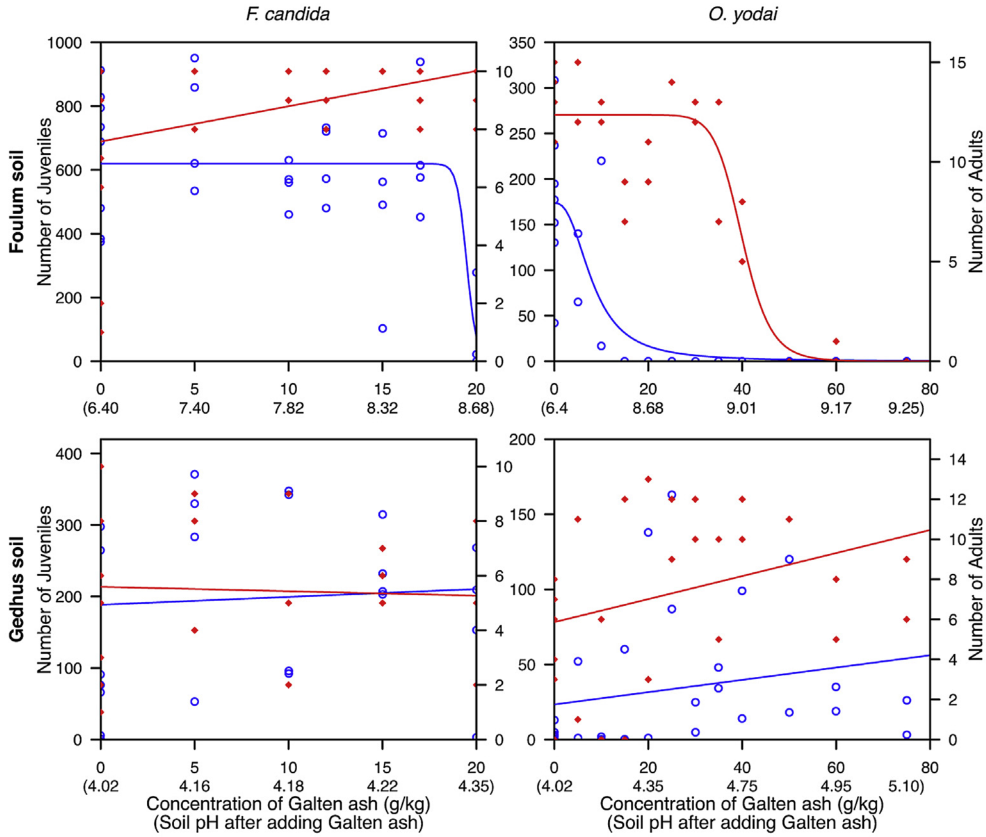

Fig. 1. Survival of adults and reproductive output, i.e. no. of juveniles per test unit, of the collembolans *F. candida* and *O. yodai* exposed to increasing concentrations of ash in an agricultural loamy sand soil from Foulum and an organic soil from the O-layer of a spruce forest at Gedhus. ○: Reproduction; ◆: Survival.

Table 4
Population toxicology endpoints of *F. candida* and *O. yodai* exposed to ash and $Ca(OH)_2$, i.e. liming, in a loamy sand, $g \cdot kg^{-1}$. Endpoints are given both in terms of ash and $Ca(OH)_2$ concentrations and in terms of soil pH measurements. 95% confidence limits in brackets. LC_{10}, LC_{50}, EC_{10} and EC_{50}: concentrations causing 10% and 50% decrease in mortality or reproduction. NOEC and LOEC: No Observed Effect Concentration and Lowest Observed Effect Concentration.

	Species	Concentration of ash, g/kg						
		LC_{10}	EC_{10}	LC_{50}	EC_{50}		NOEC	LOEC
Galten Ash	*F. candida*	-[a]	18.9 [-2.43–40.3]	–	19.5 [-3.82–42.8]	Survival	–	–
						Reproduction	17	20
	O. yodai	33.9 [28.6–39.2]	3.38 [-3.17–9.93]	40.0 [36.8–43.2]	8.15 [0.608–15.7]	Survival	10	15
						Reproduction	10	15
Ca(OH)2	*O. yodai*	5.3 [5.2–5.4]		6.9 [6.3–7.5]		Survival	5	10
Galten Ash		35 [7.6–62]		76 [56–96]		Survival	40	50
Brande Ash		40 [33–46]		73 [67–79]		Survival	30	40
	Species	Soil pH						
		LC_{10}	EC_{10}	LC_{50}	EC_{50}		NOEC	LOEC
Galten Ash	*F. candida*	-[a]	8.16 [-17.4–33.8]	–	8.43 [-22.6–39.4]	Survival	–	–
						Reproduction	8.32	8.68
	O. yodai	8.91 [8.82–9.01]	7.15 [5.96–8.34]	9.01 [8.96–9.06]	7.77 [7.16–8.37]	Survival	7.82	8.32
						Reproduction	7.82	8.32
$Ca(OH)_2$	*O. yodai*	8.11 [8.09–8.14]		8.45 [8.28–8.61]		Survival	8.05	8.33
Galten Ash		8.52 [7.92–9.12]		8.91 [8.79–9.03]		Survival	8.54	8.73
Brande Ash		8.54 [8.46–8.61]		8.85 [8.81–8.90]		Survival	8.26	8.45

[a] No significant differences between concentrations.

compared to the acidic control soil. *E. crypticus* avoids strongly acid soil (pH below 4.0) (Jänsch et al., 2005). High numbers of juveniles were found at pH values of 4.8–6.5, but the optimum pH is between 5.9 and 6.5 (Brüggl, 1994). Lower numbers were found in soils with pH values less than 4.8 and below a soil pH of 4.0 almost none was found (Jänsch et al., 2005). Higher soil pH values (7.7) only had slight effects (Achazi et al., 1997, 1996), which might be the reason for ash having no stress effect on *E. crypticus* in loamy sand. In contrast to our study Haimi et al. (2000) showed a decrease in the abundance of the only enchytraeid species, *Cognettia sphagnetorum*

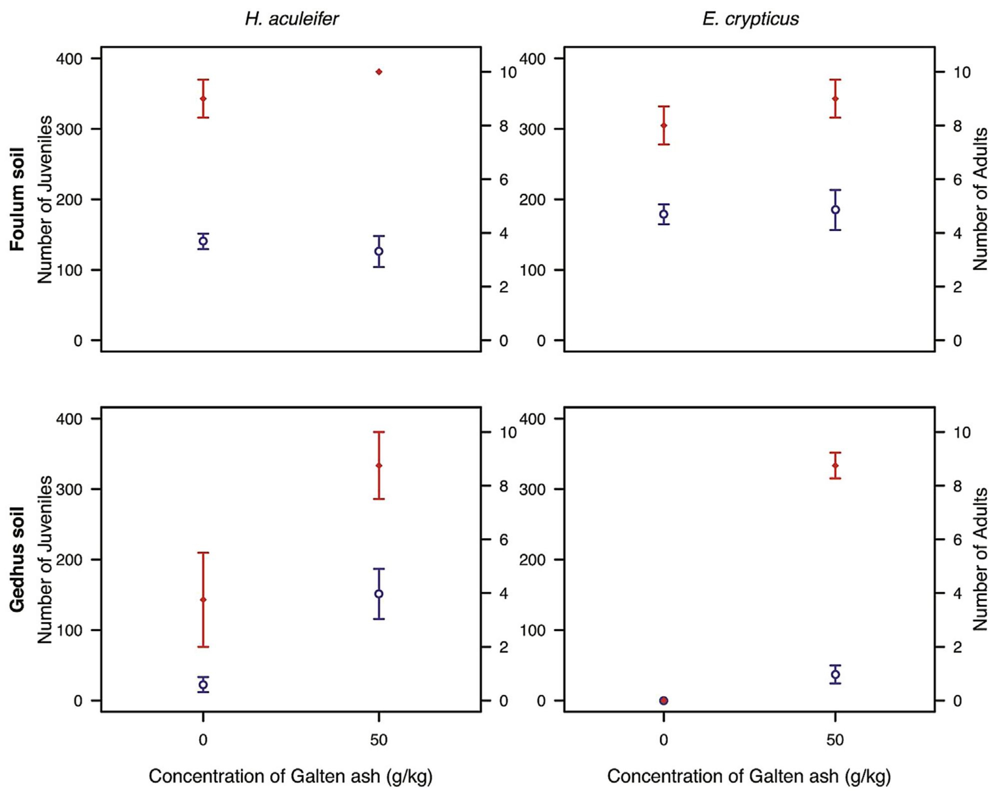

Fig. 2. *E. crypticus* and *H. aculeifer* exposed to different soil concentrations of ash. ○: Reproduction; ◆: Survival. Vertical lines are standard errors of the mean.

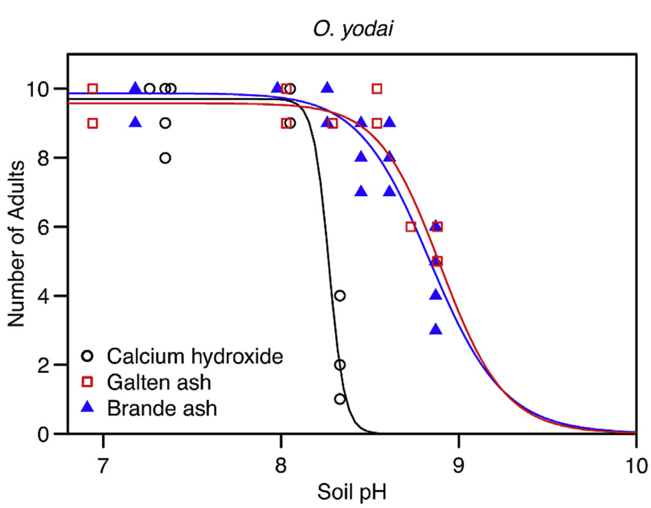

Fig. 3. Surviving adult *O. yodai* exposed to increasing concentrations of $Ca(OH)_2$, wood ash and Brande ash in an agricultural loamy sand soil. Curves are fitted to sigmoid models to the observed number of survivors.

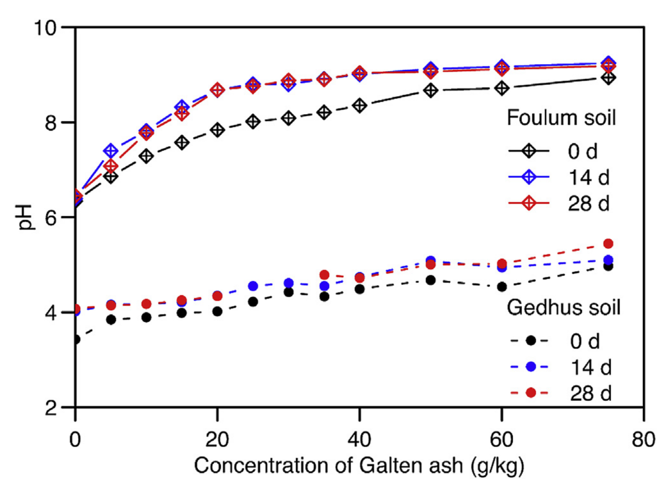

Fig. 4. Soil pH with increasing concentrations of Galten ash in an agricultural loamy sand soil (Foulum soil) and in the forest floor litter horizon (Gedhus soil).

in a coniferous forest applied 5 t ash ha^{-1}. Thus, *C. sphagnetorum* may be tolerant to low pH only and not to alkaline conditions (Huhta, 1984; Räty and Huhta, 2003). Coniferous forest stands are natural habitats of *C. sphagnetorum*, while the acidic organic soil horizon from Gedhus plantation are not suitable for *E. crypticus* as demonstrated by the increase in abundance with ash amendment compared to the control.

4.3. Collembolans in forest floor soil

We did not observe statistically significant effects on the survival and reproduction of *F. candida* and *O. yodai* when exposed to ash in Gedhus forest floor material from the O-horizon. In the microcosm study with coniferous forest floor humus and litter by Liiri et al. (2002), there were neither any significant differences on total number of microarthropods in the ash treatment, 5 t ha^{-1}, compared to the control. Although a decrease at the species level could happen, soil microarthropods are rather unaffected by ash

(Liiri et al., 2002). When ash was applied to the floor of a Scots pine stand, the treatments (1 t ha^{-1} and 5 t ha^{-1}) had no influence on collembolans (Haimi et al., 2000). However, granulated wood ash did unexpectedly increase the abundance of collembolans in spruce forest (Nieminen et al., 2012). Nieminen et al. (2012) showed that the ash effects on soil organisms were better explained by increased soil moisture than by pH and conductivity. In our study, the soil moisture was maintained at fifty percent of the WHC of the mixture of soil and ash that explained wood ash didn't show the similar effect on collembolans with previous study.

4.4. Collembolans in loamy sand

Adding ash inhibited the reproduction of *F. candida* and *O. yodai* and also decreased the survival of *O. yodai* in our loamy sand test soil. Sahana and Joy (2013) also found that fly ash significantly inhibited survival, fecundity and moulting of *Cyphoderus javanus* (Collembola) at rates of 200 g kg^{-1} sandy loam lateritic soil (50 t ash ha^{-1}) in microcosms. In our study, the effect caused by ash in terms of LOEC started at 20 g kg^{-1} (23.2 t ha^{-1}) for *F. candida* and 15 g kg^{-1} (17.4 t ha^{-1}) for *O. yodai*, which are much lower than the reported LOEC levels observed by Sahana and Joy (2013) of 250 g fly ash kg^{-1} soil for fecundity in microcosms and 50 t ha^{-1} for field populations. The lab conditions reported by Sahana and Joy (2013) did not include Baker's yeast as a food resource and this may lead to a less sensitive test system as the toxic response will be more pronounced when the potential reproduction of the test is high. A study by Scott-Fordsmand and Krogh (2004) showed that *Folsomia fimetaria* (Collembola) in a treatment with food had a considerably higher reproductive output than in a treatment without food.

Soil alkalinity appeared to be an essential factor in explaining the changes observed by Vilkamaa and Huhta (1986). In the field study by Sahana and Joy (2013) application of 50 t ha^{-1} and 200 t ha^{-1} of fly ash revealed a concentration-dependent and persistent decline in the density and relative abundance of Collembola populations. In the toxicity test and the liming test, Galten ash and Brande ash gave similar $LC_{50\text{-}pH}$, around 9. This indicates that the effect from wood ash was caused by the pH increase. Greenslade and Vaughan (2003) tested pH from 3.47 to 8.03 on collembolans in a standard laboratory test system (OECD, 1984). They found that soils with pH values between 5.4 and 6.6 were optimal for the reproduction and there was a strong decrease in reproduction at pH values greater than 7 (Greenslade and Vaughan, 2003; Jänsch et al., 2005). In the present study the pH of loamy sand increased from 6.28 to 9.17 (Fig. 3) after adding wood ash. The NOEC value calculated in terms of pH was 7.78, which agrees with Greenslade and Vaughan (2003) indicating that a pH increase can produce a strong effect, which we observed in the test with *O. yodai* with liming and the two similar types of wood ash from Galten and Brande ash causing similar pH changes. They also caused similar effect levels on the survival of *O. yodai*. It also indicated that the pH increase and the ash effect on collembolans might be related.

However, when the effect of the ashes was assumed to be singly due to a pH effect, by experimental exposure to increasing $Ca(OH)_2$ concentrations, the effect was much stronger than the effect of the ashes (Fig. 3). The LC_{50} of $Ca(OH)_2$ converted to osmolality was 1565 mOsm kg^{-1} soil pore water, while the haemolymph osmolality of soil arthropods, such as Collembola, is usually around 300 mOsm kg^{-1} (Bayley and Holmstrup, 1999). EC_{50} is 833 mOsm kg^{-1} in a reproduction test of road salt sodium chloride (Addison, 2002). 1534 mOsm kg^{-1} also caused a significant decrease in tests of salinity effects (Owojori et al., 2009). This indicates that osmolality higher than 833 mOsm kg^{-1} affects collembolans. If calcium hydroxide, the main component of ash, dissolves completely in soil pore water, Galten ash and Brande ash would be 1313 mOsm kg^{-1} and 1155 mOsm kg^{-1} respectively at LC_{50} ash concentration level. As the ash could release various ions to soil pore water, the real osmolality caused by the wood ash might be even higher than this, which means that the real osmolality brought by the wood ash was similar to $Ca(OH)_2$ at the LC_{50} levels. The effects of pH were different for $Ca(OH)_2$ and the wood ashes, while the osmotic concentrations causing the stress were similar. Therefore, we suggest that the osmotic stress was more crucial than a pH effect.

Additional factors, i.e. dioxins, may also be responsible for the results obtained, which is difficult to rule out (Boiteau et al., 2012; Norström et al., 2012; Pitman, 2006; Salmon et al., 2002).

4. Conclusions

We tested four taxonomically distant representatives of the soil mesofauna fungivores, detrivores and a predator inhabiting different niches, thus our ecological effects assessment presented here is robust. Wood ash did not have negative effects for neither survival nor reproduction of *F. candida*, *O. yodai*, *H. aculeifer* and *E. crypticus* in the test in organic soil. Wood ash applied to agricultural soil had statistical significant effects for both reproduction and survival for both collembolan species at concentrations above 15 g kg^{-1} (17.4 t ha^{-1}). The mode-of-action was the $Ca(OH)_2$ liming effect in the agricultural soil. Unidentifiable factors in the ash made the toxicity of ash less toxic compared to the pH liming effect. The main factor might be the different osmolality created by $Ca(OH)_2$ and the ashes. Moreover, wood ash effects depend on the soil type, as the effect on collembolans differed between the Foulum agricultural loamy sand and the Gedhus organic litter/humus soil. We suggest that the ash effect is mainly caused by osmotic stress.

Acknowledgement

We thank Mette Hansen for sharing information on the Gedhus soil property. This work was partly funded through the ASHBACK project funded by the Danish Council for Strategic Research. Jiayi Qin was partly funded by the Graduate School of the Science and Technology faculty at Aarhus University (GSST) through a Screening Grant and a PhD grant.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.02.041.

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