The relative importance of the bacterial pathway and soil inorganic nitrogen increase across an extreme wood-ash application gradient

Vestergård, Mette; Bang-Andreasen, Toke; Buss, Sebastian Micki; Cruz Paredes, Carla; Bentzon-Tilia, Sara; Ekelund, Flemming; Kjøller, Rasmus; Mortensen, Louise Hindborg; Rønn, Regin

Published in:
Global Change Biology. Bioenergy

DOI:
10.1111/gcbb.12494

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Vestergård, M., Bang-Andreasen, T., Buss, S. M., Cruz Paredes, C., Bentzon-Tilia, S., Ekelund, F., ... Rønn, R. (2018). The relative importance of the bacterial pathway and soil inorganic nitrogen increase across an extreme wood-ash application gradient. DOI: 10.1111/gcbb.12494

Download date: 20. Jul. 2018
The relative importance of the bacterial pathway and soil inorganic nitrogen increase across an extreme wood-ash application gradient

METTE VESTERGÅRD¹, TOKE BANG-ANDREASEN¹,², SEBASTIAN MICKI BUSS¹, CARLA CRUZ-PAREDES¹, SARA BENTZON-TILIA¹, FLEMMING EKELUND¹, RASMUS KJØLLER¹, LOUISE HINDBORG MORTENSEN¹ and REGIN RØNN¹,³,⁴

¹Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark, ²Environmental Microbiology and Biotechnology, Department of Environmental Science, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark, ³Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road Xiamen 361021, China, ⁴Arctic Station, University of Copenhagen, P.O. Box 504, 3953, Qeqertarsuaq, Greenland

Abstract

Ash from combustion of biofuels, for example wood chips, is often deposited as waste, but due to its high content of essential plant nutrients and alkalinity, it has been proposed to recycle ash as a fertilizer and liming agent in biofuel production forest. However, current legislation sets rather strict limitations for wood-ash application in biofuel production systems. The soil microfood web, that is microorganisms and their microfaunal grazers, protozoa and nematodes, is pivotal for essential ecosystem processes such as decomposition and plant nutrient release. Therefore, a thorough assessment of the impacts on microfood web structure and functioning must precede actions towards raising the currently allowed application rates. In a Danish Norway spruce plantation, we evaluate the impact of wood ash applied at dosages from 0 to the extreme case of 90 t ash ha⁻¹ on the microfood web, the bacterial community structure, soil content of inorganic nitrogen, organic matter, dissolved organic carbon and nitrogen. Using structural equation modelling (SEM), we disentangled the direct effect of the disturbance imposed by ash per se, the associated pH increase and changes in prey abundance on individual organism groups in the microfood web. The SEM showed that the pH rise was the main driver of increasing abundances of culturable heterotrophic bacteria with increasing ash doses, and via trophical transfer, this also manifested as higher abundances of bacterial grazers. Fungal-feeding nematodes were unaffected by ash, whereas carnivorous/omnivorous nematodes decreased due to the direct effect of ash. Increasing ash doses enhanced the difference between bacterial communities of control plots and ash-amended plots. The ash-induced stimulation of culturable heterotrophic bacteria and bacterial grazers increased inorganic nitrogen availability at ash doses of 9 t ha⁻¹ and above. Hence, raised limits for ash application may potentially benefit tree growth via enhanced N mineralization activity of the soil food web.

Keywords: bacterial community structure, high-resolution melt curve (HRM) analysis, inorganic nitrogen, nematodes, Norway spruce, protozoa, structural equation model (SEM)

Received 15 June 2017; revised version received 28 November 2017 and accepted 28 November 2017

Introduction

Wood ash – waste product or a valuable fertilizer?

Renewable energy sources such as wood chips play an increasing role in the global energy production. For instance, the European Council has set a mandatory target of 20% renewable energy of the total energy consumption by 2020 (COM, 2006). Long-term sustainable use of wood biomass for energy production calls for optimized forest management, where the nutrient export at harvest is balanced by similar nutrient inputs.

As wood ash contains high amounts of plant nutrients, an obvious strategy is to apply the wood ash as a biofertilizer in biofuel production plantations (Jacobson et al., 2014). The major components of wood ash are calcium, potassium, magnesium and phosphorus, while it contains virtually no nitrogen (N) (Demeyer et al., 2001). Therefore, the efficiency of ash as a fertilizer depends on the existing N in the system and/or the rate of N deposition. In spite of the negligible N content in wood ash, the increased pH can indirectly increase microbial activity and thus N mineralization and availability.

Correspondence: Mette Vestergård; tel. +45 51827047, fax +45 35322128, e-mail mevmadsen@bio.ku.dk

© 2017 The Authors. Global Change Biology Bioenergy Published by John Wiley & Sons Ltd. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
et al. (2003). Studies on the Cd effect on soil invertebrates after ash application are scarce, but no toxic effects have been observed (Lundkvist, 1998; Lodénius et al., 2009).

Thorough assessments of wood-ash application impacts on the forest ecosystem must also include detailed evaluation of effects on microorganisms and higher trophic levels of the soil food web as well as key microbial functioning.

**The importance of the soil microfood web for decomposition and nutrient mineralization**

The soil microfood web, including microorganisms and their microfaunal grazers, protozoa and nematodes (Dam et al., 2017), plays an extremely important role for key ecosystem processes, for example, decomposition and mineralization of nitrogen and other plant nutrients and hence plant growth (Rønn et al., 2012). Protozoa and nematodes are the primary microbial grazers in terrestrial ecosystems. They regulate the size and functioning of fungal and bacterial populations (Ingham et al., 1985; Rønn et al., 2012) in the soil, and nematode and protozoan grazing enhance microbial activity and nutrient mineralization (Rønn et al., 2012). Nematodes are represented at several trophic levels of the soil decomposer food web; that is bacterivorous, fungivorous, omnivorous and carnivorous taxa (Yeates et al., 1993), and the abundance of these individual feeding groups often mirrors growth responses of their respective food sources (Ferris, 2010; Christensen et al., 2012). Transfer of energy and elements in the soil food web is thought to be compartmentalized into two separate pathways that are either based on bacterial or fungal production (Moore & Hunt, 1988; Moore et al., 1996). Especially lower order consumers in the soil food web such as protozoa and nematodes reflect the carbon flow through the bacterial or fungal channel (Rooney et al., 2006).

**Ash-induced effects on the soil microfood web**

The pH increase caused by ash can have profound effects on microbial composition and productivity. In general, bacterial growth peaks at higher pH than fungal growth (Rousk et al., 2010a; Rousk & Bååth, 2011; Cruz-Paredes et al., 2017), and often increasing pH benefits bacterial abundance and productivity, whereas fungal productivity usually declines or remains unaffected (Majdi et al., 2008; Rousk et al., 2009, 2011). Shifts towards bacterial relative to fungal decomposition activity are likely to enhance mineralization rates and diminish soil C storage capacity (Waring et al., 2013; Malik et al., 2016). In general, soil pH is a principal driver of soil bacterial community composition (Fierer & Jackson, 2006; Rousk et al., 2010b); hence, also the composition of...
bacterial communities may respond to wood-ash application (Perkiömäki & Fritze, 2002; Noyce et al., 2016; Bang-Andreasen et al., 2017; Cruz-Faredes et al., 2017).

We know much less about the effects of increased pH on organisms at higher trophic levels of the soil food web. Further, there is a striking paucity of data on the effects of ash application and pH increase on protozoa, and results on the effects of wood-ash and lime application on nematodes in acidic forest soils are somewhat contradictory. Ash and lime application enhanced populations of bacterial-feeding nematodes in Finnish pine forest soils (Hyvönen & Huhta, 1989; Nieminen, 2009), whereas ash had a transient negative effect on fungal-feeding nematodes, but no effect on bacterial-feeding nematodes in a laboratory experiment with similar soil (Liiri et al., 2007). Further, defaunated soil that was buried in acidic pine forest plots attained higher abundances of microbial-feeding nematodes, but lower abundances of omnivorous nematodes after colonization from the surrounding forest soil that had been treated with wood ash than from unamended soil (Liiri et al., 2002). The variable responses may reflect that nematode responses were assessed under variable experimental conditions and may also reflect variable responses of prey organisms or co-occurring microbivores under the different experimental conditions.

Soil organisms will not only experience pH changes, but also the general system disturbances imposed by ash application, for example reduced moss coverage and nutrient and heavy metal addition (Huotari et al., 2015). Especially long-lived nematode taxa with long life cycles and slow reproduction, most of which are higher-order consumers such as omnivores and predators, are sensitive to environmental disturbance (Bongers, 1990; de Goede & Dekker, 1993).

Testing ash dose effects in a spruce forest

Our aim is to assess the effects of ash application across a wide range of doses on the bacterial community structure and the microfood web that is based on microbial production in a spruce plantation, which represents a system where enhanced application of wood ash as fertilizer is most relevant. Further, we will evaluate if ash-driven changes of the bacterial community structure and the microfood web have implications for the inorganic nitrogen availability. We also relate responses of the organisms and inorganic nitrogen availability to ash effects on soil organic matter content and labile organic substrates, that is dissolved organic carbon and nitrogen. To our knowledge, no studies report the concerted response of the major groups of the soil microfood web; that is microorganisms, protozoa and nematode trophic groups.

Effects of the application of potentially environmentally harmful products are best evaluated in dose-response trials that also include very high or extreme treatment doses. Extreme treatments increase the likelihood of detecting potential negative effects of the application of a given substance or product; vice versa, lack of effects even at extreme doses allows us to conclude with confidence that a product does not elicit harmful effects on investigated variables. Such trials are rarely conducted at field scale, but here we designed a field experiment with a gradient of ash application doses including currently allowed and extremely high doses; that is, doses that far exceed the doses that would realistically be applied in forestry.

Within recent years, the use of structural equation modelling (SEM) has remarkably advanced our causal understanding of the shifts in belowground food webs and ecological processes associated with environmental changes (Eisenhauer et al., 2013; Vestergård et al., 2015). With SEM, we will analyse whether treatment effects on abundances of culturable heterotrophic bacteria, protozoa and nematode feeding groups can primarily be ascribed to the ash application per se, to the direct effect of the associated pH raise or to ash- or pH-induced changes in the abundance of prey organisms.

For SEM, the analyst constructs a hypothesized network of variables including hypothesized causal relationships between variables. SEM is a tool that statistically evaluates the probability of the hypothesized network including the implied causal relationships between variables (McCoach et al., 2007). Therefore, significant correlation between variables does not as such prove causality (Kazantis et al., 2001). One limitation for the inference of causality between variables is that it is rarely, if ever, possible to identify and incorporate all relevant causes for a given variable (McCoach et al., 2007), and causality inference should always be interpreted with some caution.

We hypothesized that the liming effect of wood ash was the principal driver of soil organism responses to ash application, and that the pH raise would enhance the abundance of culturable heterotrophic bacteria with cascading positive effects on bacterial consumers and possibly carnivorous/omnivorous nematodes. In contrast, we expected no or a modest negative response of the fungal-based compartments of the soil food web to the pH increase. Alternatively, we hypothesized that differential sensitivity to environmental disturbances at higher trophic levels could decouple the responses of microbial grazers or higher-order consumers of the microfood web from the anticipated bacterial productivity increase at wood-ash application. Further, we hypothesized that the bacterial community composition would respond to the pH increase in a dose-dependent
manner, and we expected enhanced soil inorganic nitrogen content with enhanced microbial activity at increasing ash application doses.

Materials and methods

Field experiment and sampling

Our field site is a Norway spruce ('Picea abies' (L.) Karst.) plantation located in Mid Jutland, Denmark (N 56°16.633', E 9°05.200'), 51 m above sea level. The climate is temperate with a mean annual temperature of 8.4 °C and a mean annual precipitation of 850 mm. The plantation is a second-generation plantation established in 1957 on former heathland with a well-developed podzolization formed on a well-drained, sandy glacial till. The ground vegetation is heavily dominated by mosses with very limited contribution of vascular plants, mainly Deschampsia flexuosa and Vaccinium vitis-idaea.

The experiment was laid out as a randomized block design with six levels of wood-ash application in five replicate rows, that is in total 30 2 × 2 m plots, within a 20 × 15 m area. In April 2014, we applied the equivalent of 0, 3, 9, 15, 30 or 60 t ha⁻¹ wood ash to the individual plots, where 3 t ha⁻¹ represents the currently maximum allowed dose. The wood ash was a mixture of bottom- and fly ash originating from the burning of spruce bark chips at the nearby Brande heating plant. The ash had not been exposed to any stabilization treatment. Details of wood-ash contents and alkalinity are shown in Table 1.

In April 2015, we sampled all plots. In each 2 × 2 m plot, three samples randomly positioned at least 30 cm from the edge of the plot were taken with a 5 cm auger to a depth of 5 cm including the moss (when present), O horizon and the top of the A horizon (3 × 98 cm⁻² per plot). The samples were transported to the laboratory under cooled conditions and kept at 4 °C until processed the following day.

Sample processing and determination of physicochemical parameters

The three subsamples from each plot were pooled into one composite sample per plot.

We sieved (5 mm) and homogenized the individual composite samples. All variables were measured on fractions of the homogenized composite samples.

pH was measured on 10 g (fw) soil suspended in 25 mL distilled H₂O on a Phm 240 pH/ION meter. For soil water determination, we measured the weight loss of 5 g (fw) soil after 24 h drying at 110 °C, and subsequently we determined soil organic matter (SOM) content as mass loss on ignition at 550 °C for 6 h.

For dissolved organic carbon (DOC), dissolved organic nitrogen (DON), ammonium (NH₄⁺) and nitrate (NO₃⁻) determination, we extracted 5 g (fw) soil in 50 mL distilled H₂O for 60 min on a shaker at 200 rpm. The extracts were filtered and immediately frozen at −18 °C. We measured the DOC content in the filtrates on a Shimadzu TOC5000A analyser (Shimadzu, Kyoto, Japan). DON contents in digested filtrates and NH₄⁺ and NO₃⁻ contents in the filtrates were measured with a flow injection analyser (5000 FIASTAR, Höganäs, Sweden).

Table 1 Elemental content (ppm dw) and pH of the wood ash applied in the field experiment

| Element | Al   | As  | Ba  | Ca  | Cd  | Co  | Cu  | Cr  | Fe  | K   | Mg  |
|---------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ppm dw  | 6183 | 1.8 | 986 | 116024 | 7.1 | 4   | 88  | 14  | 5289 | 29  | 115 | 15341 |
| Mn      | 9689 | 1   | 4963 | 18  | 14682 | 29  | 666 | 0.4 | 13  | 639 | 9   | 12.95 |

© 2017 The Authors. Global Change Biology Bioenergy Published by John Wiley & Sons Ltd., 10, 320–334
methods. Samples were extracted for 72 h, and individuals of nematode trophic groups were counted while live based on morphological characteristics of mainly the buccal cavity and esophagus (Yeates et al., 1993; Dam et al., 2017) using an inverted microscope at 200× to 400× magnification. We focus on abundances of nematodes that feed on bacteria and fungi or are carnivores or omnivores, as the quantitative responses of these trophic groups often reflect growth responses of their respective food sources (Rooney et al., 2006; Ferris, 2010; Christensen et al., 2012).

**Fingerprinting of the bacterial community structure**

DNA was extracted from 3 g of frozen soil with the PowerMax Soil® kit (MoBio, Carlsbad, USA). Differences in bacterial community structure between samples were examined using real-time PCR combined with high-resolution melt curve analysis of the amplified DNA, as described by (Hjelmsø et al., 2014). PCRs were run on a real-time Bio-Rad T100 Thermal Cycler in technical triplicates using a master mix consisting of 4 µL HOT FIREPol® EvaGreen® qPCR Supermix (Solis Biodyne, Ria, Estonia), 2 µL of bovine serum albumin (BSA) (20 mg mL⁻¹; BIORON, Ludwigshafen, Germany), 0.4 µL of 10 µM forward and reverse primers (354f: 5'-CCTAYGGGRBGCASCAG-3' and 806r: 5'-GGACTACNNGGGTATCTAAT-3') (Hansen et al., 2012), 1 µL DNA template and 12.2 µL sterile water to a final volume of 20 µL. Sterile water and genomic DNA from Escherichia coli K-12 (Blattner et al., 1997) were used as negative and positive controls, respectively. Thermal conditions were an initial denaturation at 95 °C for 12 min followed by 35 amplification cycles of 95 °C for 15 s, 56 °C for 30 s, 72 °C for 30 s and ending with 72 °C for 3 min. High-resolution melt curves were generated by melting the resulting 16S rRNA gene amplicons using 0.1 °C increments from a starting temperature of 72 °C and ending at 95 °C with fluorescence measurements at every temperature increment.

Melting curves were normalized to relative fluorescence units (RFU) in the melt region of the 16S rRNA gene amplicons (83.8–90.9 °C using the Precision Melt Analysis™ Software (Bio-Rad Laboratories, Richmond, CA, USA). Based on the normalized curves, we calculated the difference in RFU for each temperature step of the HRM curve. These values represent the amount of DNA melting at a given temperature, and the data provide a fingerprint of the bacterial community (Hjelmsø et al., 2014).

**Data analysis**

Our aim was to quantify the treatment impacts in the top five cm of the forest floor, where the organic matter content and hence soil density vary considerably. We therefore present all variables on a volume basis, that is cm⁻³, to accommodate comparison on a comparable basis. We analysed the relationship between the independent variables, that is ash application dose, and the response variables, that is soil pH, abundance of culturable heterotrophic bacteria, protozoa, nematode trophic groups, SOM, DOC, DON, NH₄⁺–N and NO₃⁻–N with linear and nonlinear regression models in SigmaPlot version 13.0. Where necessary, data were log-transformed to obtain normality and homoscedasticity. For variables that responded significantly to ash application, we subsequently tested the significance of pairwise differences between ash doses with Tukey’s test.

We used SEM to analyse the relationships between ash application level, pH and abundances of organisms at different trophic positions in the soil microcosm food web to examine whether treatment effects on abundances could primarily be ascribed to the ash application per se, to the associated pH raise or to ash- or pH-induced changes in the abundance of prey organisms. We arranged the measured variables in a causal network according to our prior knowledge on soil-ecological cause-effect relationships (Fig. 6). Some of the variables were log-transformed to achieve approximate normal distribution and linear relationships between variables, and some of the variables were rescaled to achieve approximately equal variances for all variables. We fitted the variables to the SEM with the software package lavaan (Rosseel, 2012) in R (R Core Team, 2014).

We analysed overall differences in bacterial community structure with a principal component analysis (PCA), where we treated the difference in RFU for each temperature step of the HRM analysis as proxy operational taxonomic units (OTUs). We ran the PCA in PC-ORD vers. 5 (McCune & Meford, 1999).

**Results**

**Physicochemical parameters**

pH increased dramatically ($P < 0.0001$) from 3.8 in the control treatment to 7.7 at the highest ash application dose at 90 t ash ha⁻¹ (Fig. 1). The organic matter content in the upper 5 cm was quite variable within individual ash doses, but overall it decreased with increasing ash doses ($P = 0.0012$), although only two treatments, that is 3 t ash ha⁻¹ with a mean organic matter content of 1.4 g cm⁻³ and 90 t ash ha⁻¹ with a mean of 0.93 g cm⁻³, were significantly different (Tukey, $P < 0.05$) (Fig. 2a). Likewise, DOC and DON varied considerably within individual ash application levels, but both variables increased with ash doses up to 15–30 t ash ha⁻¹. At 90 t ash ha⁻¹, the variation within both labile organic pools increased even further, but there was an overall decline in DOC and DON compared to the maximum values found at lower ash doses (Fig. 2a,b).

Soil ammonium concentration increased dramatically from 1.5 to 1.7 µg NH₄⁺–N at 0 to 3 t ash ha⁻¹ to 11 µg NH₄⁺–N at 9 t ash ha⁻¹, and remained at a high level between 14 and 19 µg NH₄⁺–N at 15–90 t ash ha⁻¹ (Fig. 3). At 0 and 3 t ash ha⁻¹, nitrate concentrations were very low (0.1 µg NO₃⁻–N cm⁻³), but it increased linearly ($P < 0.0001$) with ash doses to 6 µg NO₃⁻–N cm⁻³ at 90 t ash ha⁻¹ (Fig. 3).

**Soil organism abundances**

The density of cultivable heterotrophic bacteria increased ($P < 0.0001$) more than twentyfold from the...
control plots to 15 t ash ha\(^{-1}\), whereas there was no further increase in densities from 15 to 90 t ash ha\(^{-1}\) (Fig. 4).

Similarly, densities of amoebae/flagellates \((P = 0.0006)\) and ciliates \((P = 0.006)\) increased 15 and 25 times, respectively, from 0 to 15 t ash ha\(^{-1}\) and remained at this high level from 15 to 90 t ash ha\(^{-1}\) (Fig. 4).

The abundances of bacterial-feeding nematodes were variable, but numbers increased modestly \((P = 0.015)\) and roughly doubled from the control to the highest ash dose (Fig. 5a). However, there were no significant pairwise differences in bacterial-feeding nematode abundance between individual ash application doses (Tukey \(P > 0.05\)). Fungal-feeding nematodes, on the other hand, were not affected by ash application \((P = 0.21; \text{Fig. 5a})\), and the density of carnivorous/omnivorous nematodes decreased \((P = 0.0002)\) with increasing ash application doses; their mean densities were already reduced by 50\% at 3 t ash ha\(^{-1}\) and were close to zero at 30 and 90 t ha\(^{-1}\) (Fig. 5b).

**Mechanisms of ash effects**

The SEM adequately fit the data \((\chi^2 = 5.474, \text{df} = 6, P = 0.485)\). The significant positive regression coefficient between pH and culturable heterotrophic bacteria revealed that the pH raise was driving the bacterial density increase at increasing ash doses, whereas the residual effect of ash dose was insignificant (Fig. 6). The positive pH effect on abundances of culturable heterotrophic bacteria transferred to amoebae/flagellates, as the relationship between culturable heterotrophic bacteria and amoebae/flagellates was significantly positive and further to ciliates that were positively related to amoebae/flagellates. In addition to the bacterial-mediated association between pH and amoebae/flagellates, there was also a direct positive relationship between the pH rise and abundances of amoebae/flagellates. There was no direct influence of ash or pH on bacterial-feeding nematodes, but a negative relationship between abundances of culturable heterotrophic bacteria and bacterial-feeding nematodes.

---

**Fig. 1** pH in the top 5 cm of forest floor plots 1 year after the application of no wood ash (0) or increasing doses of wood ash (3, 9, 15, 30 or 90 t ash ha\(^{-1}\)). The curve represents the fit to an exponential rise to maximum regression model, where pH = 3.7340 + 4.0103 \times (1 - e^{-0.0515 \times t \text{ ash/ha}}) with \(r^2\) and \(P\) values for the regression model. Ash doses with different letters are significantly different (Tukey \(P < 0.05\)).

**Fig. 2** (a) Soil organic matter (SOM), dissolved organic (b) C (DOC) and (c) N (DON) content in the top 5 cm of forest floor plots 1 year after the application of no wood ash (0) or increasing doses of wood ash (3, 9, 15, 30 or 90 t ash ha\(^{-1}\)). The curves represent fits to (a) an exponential decay model, where SOM = 1.3535 \times e^{-0.0049 \times t \text{ ash/ha}}, and (b,c) second-order polynomial models, where log DOC = -0.1289 + 0.0132 \times t \text{ ash ha}^{-1} - 0.0002 \times (t \text{ ash ha}^{-1})^2 and log DON = -1.3171 + 0.0154 \times t \text{ ash ha}^{-1} - 0.0002 \times (t \text{ ash ha}^{-1})^2, with \(r^2\) and \(P\) values for the regression models. Ash doses with different letters are significantly different (Tukey \(P < 0.05\)).
\[ r^2 = 0.32, \quad P = 0.0012 \]

\[ r^2 = 0.27, \quad P = 0.014 \]

\[ r^2 = 0.35, \quad P = 0.003 \]
Fig. 3  Concentrations of NH$_4^+$–N (log(µg NH$_4^+$–N cm$^{-3}$)) and NO$_3^-$–N (log(1 + µg NO$_3^-$–N cm$^{-3}$)) in the top 5 cm of forest floor plots 1 year after the application of no wood ash (0) or increasing doses of wood ash (3, 9, 15, 30 or 90 t ash ha$^{-1}$). Curves represent fits to an exponential rise to maximum regression model, where log(µg NH$_4^+$–N cm$^{-3}$) = 1.169 + 0.1461 $t$ ash ha$^{-1}$ and a linear regression model, where log(1 + µg NO$_3^-$–N cm$^{-3}$) = 0.1389 + 0.0078 $t$ ash ha$^{-1}$, respectively, with $r^2$ and $P$ values for the regression models. Ash doses with different letters are significantly different (Tukey $P < 0.05$).

Fig. 4  Abundance (number cm$^{-3}$) of culturable, heterotrophic bacteria, amoebae/flagellates and ciliates in the top 5 cm of forest floor plots 1 year after the application of no wood ash (0) or increasing doses of wood ash (3, 9, 15, 30 or 90 t ash ha$^{-1}$). The curves represent the fits to exponential rise to maximum regression models, where log(culturable heterotrophic bacteria) = 5.3853 + 1.4001 $t$ ash ha$^{-1}$, log(amoebae/flagellates) = 4.3214 + 1.407 $t$ ash ha$^{-1}$ and log(ciliates) = 2.4606 + 1.5799 $t$ ash ha$^{-1}$ with $r^2$ and $P$ values for the regression models. Ash doses with different letters are significantly different (Tukey $P < 0.05$).
Besides pH, the only variable that responded positively to ash application per se was the ciliates, and only carnivorous/omnivorous nematodes responded negatively to the direct effect of increasing ash doses.

**Bacterial community structure**

The first and second axes of the PCA explained 53.9% and 25.3% of the variance of the total variation in the high-resolution melting analysis, respectively (Fig. 7).

The first axis was highly correlated with soil pH and clearly separated bacterial communities in control plots from bacterial communities in ash-treated plots and communities in plots exposed to 3 t ash ha$^{-1}$ from communities in plots exposed to higher ash application doses (Fig. 7). Despite a tenfold increased ash application dose from 9 to 90 t ha$^{-1}$, there was no clear separation of bacterial communities subjected to ash levels exceeding 3 t ha$^{-1}$. The variation between bacterial communities within individual treatments was large for

**Fig. 5** Abundance (log number cm$^{-3}$) of (a) bacterial-feeding, fungal-feeding and (b) carnivorous/omnivorous nematodes in the top 5 cm of forest floor plots 1 year after the application of no wood ash (0) or increasing doses of wood ash (3, 9, 15, 30 or 90 t ash ha$^{-1}$). In (a), the solid line represents the fit to a linear regression model, where log(bacterial-feeding nematodes) = 1.9473 + 0.0035 × t ash ha$^{-1}$, in (b) the curve represents the fit to an exponential decay regression model, where log(1+carnivorous/omnivorous nematodes) = 0.9261 × e$^{-0.0388$×$t$ ash/ha} with $r^2$ and $P$ values for the regression models. Ash doses with different letters are significantly different (Tukey $P < 0.05$).
treatments above 3 t ha\(^{-1}\) compared with the control and 3 t ha\(^{-1}\) treatments.

**Discussion**

*pH increase is the principal driver of microfood web responses to wood-ash addition*

As hypothesized, the pH increase caused by ash application (Fig. 1) increased abundances of culturable heterotrophic bacteria and bacterivorous protozoa (Fig. 4). Even at the extremely high ash doses, the densities of culturable heterotrophic bacteria and their protozoan grazers remained at levels that were ca. 20 times higher than the densities in control plots. The increase is consistent with the increase in labile substrate (DOC and DON) for microbial growth from 0 to 30 t ash ha\(^{-1}\) (Fig. 2b,c) and confirms the correspondence between the DOC and microbial biomass response to ash addition seen in mesocosms (Pugliese *et al.*, 2014). In a microcosm experiment, the addition of high ash doses (40 t ash ha\(^{-1}\)) also enhanced the DOC and DON contents in the soil (Ludwig *et al.*, 2000). We note that microbial metabolites associated with increased microbial activity may also contribute to the increase of labile organic fractions (Hansen *et al.*, 2016). At 90 t ash ha\(^{-1}\), we measured low DOC and DON contents in some of the plots. This reflects that the mineral soil contributed relatively more to the top 5 cm of the forest floor as a consequence of the reduced SOM content at 90 t ha\(^{-1}\) (Fig. 2a).
Similarly to our findings, wood-ash application enhanced microbial activity and abundance in mesocosms with coniferous forest soil (Fritze et al., 2000) as well as bacterial-feeding nematode abundances (Niemi- nen, 2009). It is well-known that liming of acidic environments like the forest floor investigated here stimulates bacterial activity and production (Rousk et al., 2009, 2011; Cruz-Paredes et al., 2017), and Räty & Huhta (2003) found that a pH increase from 4.9 to 6.2 enhanced bacterial-feeding nematodes. The SEM analysis supported that the pH rise, and not the ash as such, was the main driver of the increased density of culturable heterotrophic bacteria (Fig. 6). The significant relationship between culturable heterotrophic bacteria and amoebae/flagellates and further between amoeba/flagellates and ciliates demonstrates that the positive effect of ash application on protozoa could be partly ascribed to the underlying pH driven increase of their prey.

Previously, it was assumed that declining densities of naked amoebae and flagellates at increased soil acidification were solely driven by the concomitant reduction of microbial prey availability (Ekelund & Renn, 1994), but the SEM suggested that the reduced acidity in itself adds to the positive effect on amoebae/flagellates. Further, the SEM revealed a direct positive coupling between ash application doses and ciliate abundance. Ciliates are known to display much lower activity than expected considering the availability of food items ('ciliotstasis') (Foissner, 1987). This is probably caused by hitherto unknown chemical substances in the soil (Ekelund et al., 2002). Certain types of disturbances break ciliotstasis; for example drying-rewetting, and components in the ash may work similarly. We also note that the general food quality of available food items probably improved with the nutrients applied with the ash.

The abundance of bacterial-feeding nematodes varied considerably within the individual ash application levels, and even though there was an overall positive relationship between ash dose and bacterial-feeding nematode abundance (Fig. 5a), this was a modest increase compared to the protozoan response.

The enhanced energy flow through the bacterial-based decomposer channel of the microfood web was not transferred to the next trophic level, that is carnivorous/omnivorous nematodes. Their densities decreased with increasing ash application doses (Fig. 5b), and the SEM showed that the negative ash effect was not related to the liming effect, rather there was a direct negative relationship between ash doses and carnivorous/omnivorous nematodes (Fig. 6). Thus apparently, differential sensitivity to wood-ash application at different trophic levels decoupled higher-order trophic levels from the enhanced productivity at the first- and second-order level of the decomposer food web. As previous investigations did not detect any Cd effects on natural...
nematode communities or typically disturbance-sensitive nematode taxa (Korthals et al., 1996; Bakonyi et al., 2003; Nagy et al., 2004), Cd added with the ash probably did not cause the decline of carnivorous/omnivorous nematodes. In our field experiment, ash application reduced and changed the species composition of the moss cover (Ethelberg-Findsen et al., in prep), and this overall habitat disturbance may explain the negative response of the most sensitive nematodes. Further, at reduced organic matter content (Fig. 2a), the average soil pore diameter declines, which can restrict the large-sized carnivorous/omnivorous nematodes from accessing prey residing in small pores spaces (Mikola & Setälä, 1998a). We speculate that the modest increase in bacterial-feeding nematode abundances with increasing ash doses may reflect reduced predatory control from the declining populations of carnivorous/omnivorous nematodes (Mikola & Setälä, 1998b). In general, populations of carnivorous/omnivorous nematodes increase slower than populations of typically more r-selected bacterial feeders (Bongers & Bongers, 1998). Here, we study responses to ash 1 year after application; the positive effect on abundances of lower trophic organisms may thus later manifest as enhanced carnivorous/omnivorous nematode densities or to some degree counteract the immediate negative effect on carnivorous/omnivorous nematodes.

Contrary to the bacterial-based compartment of the soil food web, ash application did not affect fungal-feeding nematode abundances (Fig. 5a). The abundance of bacterial- and fungal-feeding nematodes has been shown to correlate with the activity of their food sources, that is bacteria and fungi, respectively (Christensen et al., 2012). Our data thus support previous findings that fungal production responds very little, if at all, to rising pH (Rousk et al., 2009, 2011). Rousk et al. (2010b) stated that fungal diversity is largely unresponsive to soil pH changes, but there is also evidence that fungal diversity is highest in acidic soils, and soil pH is an important driver of fungal and particularly ectomycorrhizal fungal community composition (Tedersoo et al., 2014; Kjøller et al., 2017).

\[ \text{pH-related impacts on bacterial community structure} \]

There is strong evidence that pH is the main driver of bacterial community composition at both continental scales (Fierer & Jackson, 2006) and local scales (Lanzén et al., 2015; Zhalnina et al., 2015), and our analysis confirms that the wood ash-induced community change strongly correlates with pH (Fig. 7). In our system, the largest bacterial community changes were associated with pH increases from 3.8 to 5.3, that is from 0 to 9 t ash ha\(^{-1}\).

Within this range of ash doses and pH increase, our analyses thus confirm previous findings that modest ash application doses and the associated pH increase significantly alter the composition of microbial, especially bacterial, communities (Frosteåård et al., 1993; Perkkimmäki & Fritze, 2002; Noyce et al., 2016) resulting in community-wide tolerance to higher pH values (Cruz-Paredes et al., 2017). However, contrary to our expectation ash doses above 9 t ash ha\(^{-1}\) did not separate bacterial communities in a dose-dependent manner. The larger variation of bacterial community composition within treatments exposed to 9–90 t ash ha\(^{-1}\) suggests that stochastic processes to a larger extent governed the development of spatially separated bacterial communities exposed to extreme ash application doses.

The importance of pH as the driver of microbial community composition probably also explains why low wood-ash application rates of maximum 3.3 t ha\(^{-1}\) did not affect the microbial community composition in drained peatlands, where wood ash did not raise the pH (Björk et al., 2010).

Given that wood-ash application did not induce enhanced Cd tolerance in bacterial communities sampled at our field site (Cruz-Paredes et al., 2017), we consider it highly unlikely that the Cd content in the ash applied in our experiment (7.1 mg kg\(^{-1}\) ash) contributed to the changes in microbial community structure. Further, in another study, spiking wood ash with very high Cd levels (up to 1,000 mg kg\(^{-1}\) ash) did not in any way affect the microbial community composition (Fritze et al., 2000).

\[ \text{Implications of wood-ash application on inorganic nitrogen availability and soil C storage} \]

As wood ash contains practically no nitrogen, its applicability as a fertilizer critically depends on its impact on inorganic nitrogen availability, especially in N-limited systems.

We found that application of 9 t ash ha\(^{-1}\) and above increased ammonium concentrations more than 10 times, and at 30 and 90 t ash ha\(^{-1}\), mean nitrate concentrations were 18 and 41 times higher than in the control, respectively. This is in accordance with the concurrent increase of labile organic N (DON), readily available for microbial turnover (Kielland et al., 2007) and the pronounced stimulation of cultivable heterotrophic bacteria and bacterial grazers that are responsible for soil nitrogen mineralization (Rønn et al., 2012). At our study site, vascular plants contribute very little to the forest floor vegetation, which is totally dominated by mosses. Ash application reduced the moss cover, and the increased inorganic N content with ash application could thus partly reflect reduced N uptake by mosses. However,
the 10-fold increase in ammonium was already apparent at 9 t ash ha\(^{-1}\), whereas the moss cover did not decline before 15–90 t ash ha\(^{-1}\) (Ethelberg-Findsen \textit{et al.}, in prep.), and we therefore consider increased mineralization the most likely cause of the increased ammonium concentration.

In Finnish and Swedish studies, 3–4 t ash ha\(^{-1}\) either had no or low, transient positive impact on soil ammonium concentrations (Jacobson \textit{et al.}, 2004; Nieminen \textit{et al.}, 2012). At one site, ammonium concentrations in the humus layer increased ca. 2 and 4 times at 6 and 9 t ash ha\(^{-1}\), respectively, whereas at another site, ash did not affect ammonium concentrations (Jacobson \textit{et al.}, 2004). We found no increase in the inorganic N availability at 3 t ash ha\(^{-1}\). Taken together, these results suggest that consistent and lasting positive effects on ammonium availability require ash application doses above the currently allowed limits.

Whereas the NH\(_4\)\(^+-\)N pool increased dramatically already at 9 t ash ha\(^{-1}\), the NO\(_3\)\(^--\)N pool increased more moderately and gradually. Although the traditional notion that nitrification is limited in acidic soils has been challenged (Booth \textit{et al.}, 2005; Nicol \textit{et al.}, 2008), our study shows that significant nitrate production is not evident before near-neutral pH is reached.

Our plots of 2 × 2 m are too small to relate the increased inorganic N availability to tree N uptake and growth, which is key to assessing the economic and environmental sustainability of ash application, but positive effects of ash application on tree production have been reported for sites on organic soils (Karltun \textit{et al.}, 2008; Huotari \textit{et al.}, 2015).

The main rationale for the use of wood as an energy source is the replacement of fossil fuel and the conversion of energy production towards systems where CO\(_2\) uptake during biofuel production compensate for CO\(_2\) emissions. However, if ash-induced mineralization of soil C exceeds the CO\(_2\)-C fixed during tree growth the climate change mitigating rationale may prove untenable. Therefore, on the one hand, the ash-induced increased activity of the bacterial-based decomposer food web jeopardizes the sustainability of biofuel production by increasing soil organic matter decomposition, but on the other hand, the concomitant N mineralization may increase tree growth and thus CO\(_2\) fixation. Further research is pertinent to clarify, if the ash-induced enhanced inorganic N content will positively impact tree growth and adequately compensate for ash-induced mineralization of soil organic C.

In conclusion, wood-ash application increased labile organic pools and the production of culturable heterotrophic bacteria in the top 5 cm of the forest floor with cascading effects on the bacterial-feeding trophic level. This response was governed by the limiting effect of wood ash rather than by ash per se. In contrast, ash application per se, rather than the associated pH change, reduced abundances of carnivorous/omnivorous nematodes, confirming that nematode taxa at this trophic level are sensitive to environmental disturbances.

Further, wood-ash application and the associated pH increase changed the bacterial community structure, but the changes induced by the most extreme ash dose of 90 t ash ha\(^{-1}\) did not exceed the changes induced at 9 t ash ha\(^{-1}\).

As expected, the stimulation of bacterial-based micro- food web enhanced inorganic nitrogen availability. At extreme ash doses, soil organic matter decreased, but at 0−15 t ash ha\(^{-1}\) the soil organic matter pool was unaffected. Overall, the negative effects of ash application on abundances of soil biota were very limited, and the microfood web functioning proved robust to dosages exceeding currently allowed doses up to 30 times. Hence, we do not foresee negative implications for the ecosystem parameters investigated here, if legislative limits for ash application doses are raised to, for example, 6−9 t ash ha\(^{-1}\).

Acknowledgements

This work was supported by the ‘Center for Bioenergy Recycling – ASHBACK’ funded by the Danish Council for Strategic Research (grant no. 0606-00587B). We thank the forest owner, Steen Blicher A/S, for allowing us to establish the field study and ASHBACK collaborators for establishing and maintaining the field experiment. We appreciate the comments of three anonymous reviewers that significantly improved the manuscript.

References

Bakonyi G, Nagy P, Kádár I (2003) Long-term effects of heavy metals and microelements on nematode assemblage. Toxicology Letters, 140–141, 391–401.
Bang-Andreasen T, Nielsen JT, Vorsikova J, Heise J, Rønn R, Hansen HCB, Jacobsen CS (2017) Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. Frontiers in Microbiology, 8, 1400.
Blattner FR, Plunkett III G, Bloch CA (1997) The complete genome sequence of \textit{Escherichia coli} K-12. Science, 277, 1405–1432.
Bongers T (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83, 14–19.
Bongers T, Bongers M (1998) Functional diversity of nematodes. Applied Soil Ecology, 10, 239–251.
Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs, 75, 139–157.
Christensen S, Dam M, Vesteråård M, Petersen SO, Olesen JEL, Schjønning P (2012) Specific antibiotics and nematode trophic groups agree in assessing fungal-bacterial activity in agricultural soil. Soil Biology & Biochemistry, 55, 17–19.

© 2017 The Authors. Global Change Biology Bioenergy Published by John Wiley & Sons Ltd., 10, 320–334
COM (2006) COM 2006/848: Renewable Energy Road Map. Commission of the Euro-
pean Community, Brussels, Belgium.
Cruz-Paredes C, Wallander H, Kjøller R, Rouk J (2017) Using community trait-dis-
tributions to assign microbial responses to pH changes and Cd in forest soils trea-
ted with wood ash. Soil Biology & Biochemistry, 112, 153–164.
Dans A, Bergman T, Vostergård M (2017) Elevated CO2 increases fungal-based micro-
foodwebs in soils of contrasting plant species. Plant and Soil, 415, 549–561.
https://doi.org/10.1007/s11104-017-3191-3.
DEA (2008) Bioiskeblædsforgrevelser. Danish Environmental Protection Agency, Copen-
hagen, Denmark.
Demeyer A, Vountsi Nikana JC, Vertlo MG (2011) Characteristics of wood ash and in-
fluence on soil properties and nutrient uptake: an overview. Bioresource Techno-
logy, 77, 287–295.
Eisenhauer N, Dobies T, Cesarz S, Hobbie SE, Meyer RJ, Worm K, Reich PB (2013)
Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N
deposition in a long-term grassland experiment. Proceedings of the National Academy of
Sciences of the United States of America, 110, 6889–6894.
Ekulund F, Reen R (1994) Notes on protozoa in agricultural soil with emphasis on
heterotrophic flagellates and naked amoebas and their ecology. FEMS Microbiol-
ogy Reviews, 15, 321–335.
Ekulund F, Frederiksen HB, Reen R (2002) Population dynamics of active and total
ciliate populations in arable soil amended with wheat. Applied and Environmental
Microbiology, 68, 1096–1101.
Ethelberg-Findsen D, Rønn R, Ekelund F (in prep) The community structures of prokaryotes and fungi in mountain pasture
soils are highly correlated and primarily influenced by pH. Frontiers in Microbiol-
yogy, 6, 1321.
Eliasson T, Kjellman H, Nordlund S, Sikström A (2008) Wood ash recycling:
possibilities and risks. In: Sustainable Use of Forest Biomass for Energy: A Synthesis with Focus on the
Baltic and Nordic Regions (eds Röser D, Aiskainen A, Raulund-Rasmussen K, Stupka D, pp. 79–108. Springer, Dordrecht, the Netherlands.
Kazantzis N, Doane FP, Ronan KR (2001) Concluding causation from correlation:
Comment on Burns and Spangler (2000). Journal of Consulting and Clinical Psychol-
ogy, 69, 1079–1083.
Kielland K, McFarland JW, Russ SW, Olson K (2007) Rapid cycling of organic nitrogen
in taiga forest ecosystems. Ecosystems, 10, 360–368.
Kjøller R, Cruz-Paredes C, Clemmensen K (2017) Ectomycorrhizal fungal responses
to forest liming and wood ash addition: review and meta-analysis. In: Soil Biologi-
cal Communities and Ecosystem Resilience (eds Lukac M, Grenni P, Gamboni M),
Springer International Publishing.
Korpilahti A, Mönönen M, Finer I (1998) Biomass ash utilization in Finland. In:
Ashes and Particulate Emissions from Biomass Combustion: Formation, Characterisation, Evaluation, Treatment (ed Obernberger I), University of Graz, Institute of Chemical
Engineering and Technology.
Korthals GW, ENDE AVD, Megen Hv, Kammenga JE, Borgers T
(1996) Short-term effects of cadmium, copper, nickel and zinc on soil nematodes
from different feeding and life-history strategy groups. Applied Soil Ecology, 4,
107–117.
Lanzén A, Epelde I, Garbisu C, Anza M, Martín-Sánchez I, Blanco F, Mijnongs I
(2018) The community structures of prokaryotes and fungi in mountain pasture
soils are highly correlated and primarily influenced by pH. Frontiers in Microbiol-
yogy, 6, 1321.
Lairi M, Setälä H, Haimi J, Pennanen T, Fritze H (2002) Soil processes are not influ-
enced by the functional complexity of soil decomposer food webs under distur-
bance. Soil Biology & Biochemistry, 34, 1009–1120.
Lairi M, Ilmarinen K, Setälä H (2007) Variable impacts of entchytrad worms and
ectomycorrhizal fungi on plant growth in raw humus soil treated with wood ash.
Applied Soil Ecology, 35, 174–183.
Lodenius M (2003) Cadmium concentrations in a boreal forest ecosystem after appli-
cation of wood ash. Bulletin of Environmental Contamination and Toxicology, 71,
776–781.
Lodenius M, Jossenson J, Heliovaara K, Tuinala E, Nummenlin M (2009) Cadmium in
insects after ash application. Insect Science, 16, 93–98.
Ludwig W, Bell B, Reese F (2000) Use of 13C and 15N mass spectrometry to study the
decomposition of Calamagrostis epigeios in soil column experiments with and
without ash additions. Isotopes in Environmental and Health Studies, 36, 49–
61.
Lundkvist H (1998) Wood ash effects on entchytraeid and earthworm abundance
and entchytraeid cadmium content. Scandinavian Journal of Forest Research, 13,
86–90.
Majdi H, Trous L, Johansson U, Nylund J-E, Wallander H (2008) Effects of slash
retention and wood ash addition on fine root biomass and production and fungal
mycelium in a Norway spruce stand in SW Sweden. Forest Ecology and Manage-
ment, 255, 2109–2117.
Malik AA, Chowdhury S, Schlager V, et al. (2016) Soil fungal:bacterial ratios are
linked to altered carbon cycling. Frontiers in Microbiology, 7, 1247.
McCoag DB, Black AC, O’Connell AA (2007) Errors of inference in structural equa-
tion modeling. Psychology in the Schools, 44, 461–470.
McCune B, Mefford M (1999) Multivariate Analysis of Ecological Data. Gleneden
Beach, MJM Software, Lincoln, OR, USA.
Mikola J, Setälä H (1998a) Productivity and trophic-level biomasses in a microbial-
based food web. Oikos, 82, 158–168.
Mikola J, Setälä H (1998b) No evidence of trophic cascades in an experimental
microbial-based soil food web. Ecology, 79, 153–164.
Moore JC, Hunt HW (1987) Resource compartmentation and the stability of real
ecosystems. Nature, 333, 261–263.
Moore JC, Deruiter PC, Hunt HW, Coleman DC, Freckman DW (1996) Microcosms
and soil ecology: critical linkages between field studies and modelling food webs.
Ecology, 77, 694–705.
Nagy P, Bakonyi G, Bongers T, Kádár J, Fábián M, Kiss I (2004) Effects of microe-
лементs on soil nematode assemblages seven years after contaminating an agricul-
tural field. Science of The Total Environment, 320, 131–143.
Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the
diversity, abundance and transcriptional activity of ammonia oxidizing archaea
and bacteria. Environmental Microbiology, 10, 2896–2978.
Nieminen JK (2009) Combined effects of loose wood ash and carbon on inorganic N and P, key organisms, and the growth of Norway spruce seedlings and grasses in a pot experiment. Plant and Soil, 317, 155–165.
Nieminen JK, Raisanen M, Haimi J (2012) Spot mounding and granulated wood ash increase inorganic N availability and alter key components of the soil food web in clear-cut Norway spruce forests. Forest Ecology and Management, 263, 24–30.
Noyce GL, Fullthorpe R, Gorgolewski A, Hazlett P, Tran H, Basliko N (2016) Soil microbial responses to wood ash addition and forest fire in managed Ontario forests. Applied Soil Ecology, 107, 368–380.
Olsen A, Bakken I (1987) Viability of soil bacteria: optimisation of plate-counting technique and comparison between total counts and plate counts within different size groups. Microbial Ecology, 13, 59–74.
Page FC (1988) A New Key to Freshwater and Soil Cyanophyceae. Ambleside, Freshwater Biological Association.
Perkimäki J, Fritze H (2002) Short and long-term effects of wood ash on the boreal forest humus microbial community. Soil Biology & Biochemistry, 34, 1343–1353.
Pugliese S, Jones T, Preston MD, Hazlett P, Tran H, Basliko N (2014) Wood ash as a forest soil amendment: the role of boiler and soil type on soil property response. Canadian Journal of Soil Science, 94, 621–634.
R Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Rønn R, Ekelund F, Christensen S (1995) Optimizing soil extract and broth media for MPN-enumeration of naked amoebas and heterotrophic flagellates in soil. Pedobiologia, 39, 10–19.
Waring BG, Averill C, Hawkes CV (2013) Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecology Letters, 16, 887–894.
Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgieva SS (1993) Feed-in-habits in soil nematode families and genera – an outline for soil ecologists. Journal of Nematology, 25, 315–331.
Zhalnina K, Dias R, De Quadros PD et al. (2015) Soil pH determines microbial diversity and composition in the Park Grass experiment. Microbial Ecology, 69, 395–406.