Identifying potential antioxidant compounds in NaOH extracts of UK soils and vegetation by untargeted mass spectrometric screening

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ABSTRACT

NaOH extracts of soil and vegetation have been shown to have antioxidant capacity (AOC). However, the identification of specific antioxidant compounds within extracts has proved to be difficult. In earlier work we demonstrated that the presence of some lignin-derived phenolic compounds, which were antioxidants, could only account for a very small percentage of the overall antioxidant capacity of soil extracts. The aims of the work reported here were to measure AOC of both vegetation and soil extracts by the Trolox equivalent antioxidant assay and photochemiluminescence and to analyse them using pyrolysis-field ionisation mass spectrometry (Py-FIMS), which allows a tentative assignment of specific compounds and an amalgamation to broad groups of compounds (e.g. carbohydrates) for the soil extracts. With this information we could find which compounds were closely related to the AOCs of the extracts and of those which might potentially be antioxidants. We extracted six soil samples, which had a range of antioxidant capacities, and the vegetation associated with those soils. The AOC results by the two methods were well correlated, and comparing soil and vegetation AOCs suggested that some antioxidants in vegetation were lost during humification, and that some others were soil-derived. For the soil samples we found that, because most of the compound groups quantified by Py-FIMS were correlated with the antioxidant capacities, a clear assignment of antioxidant properties to groups was restricted. However, individual compounds could be linked to the AOC using multivariate statistics, grouping the samples by their antioxidant capacity. Some compounds were present in both soil and vegetation, and presumably survived humification, while others were only found in the soil, in which they had probably been formed. Further investigation of these tentatively identified compounds provides a way forward in explaining the AOC of soil and vegetation extracts.

1. Introduction

It was hypothesised by Rimmer (2006) that antioxidants are present in soil and that they are involved in the protection of soil organic matter (SOM) from oxidation. This was supported by Aeschbacher et al. (2012) who quantified the electron donating capacities of humic substances and natural organic matter by a mediated electrochemical oxidation approach in relation to redox potential and pH. They concluded that these heterogeneous organic macromolecules contain phenolic electron-donating moieties,

which may act as antioxidants. It has been demonstrated that antioxidants can be extracted from soils with NaOH, and that the quantities varied from soil to soil in proportion to their organic carbon contents (Rimmer and Smith, 2009; Cardelli et al., 2012). In addition Cardelli et al. (2012) concluded that AOC appeared to influence the rate of organic C mineralization more than relative contents of the easily mineralizable C pools. Rimmer and Abbott (2011) measured the amounts of specific lignin-derived phenolic compounds in NaOH extracts of soil, expecting that the antioxidant capacities (AOCs) of some of these compounds would be major contributors to the overall AOC of the extract. However, they only contributed to a very small percentage to the overall AOC. Thus other extracted antioxidant compounds must be present. In order to identify those other compounds, we have employed pyrolysis-field ionisation mass spectrometry (Py-FIMS) in the present study.

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Py-FIMS is an untargeted, highly sensitive and versatile analytical method, which detects all organic molecules, provided that they are volatilized in high vacuum between ambient temperature and 700 °C and ionised in the high electric field (Schulten and Leinweber, 1996). The method was successfully applied to characterise the molecular–chemical composition of dissolved organic matter (Schulten et al., 2002) and bulk soil samples (Sorge et al., 1993a). Individual m/z signals or series of signals and their thermal volatilization curves recorded by Py-FIMS explained, e.g. diurnal cycles of rhizodeposition (Melnitchouck et al., 2005; Schlichting and Leinweber, 2009), the binding capacity of SOM for antibiotics (Thiele-Bruhn et al., 2004), enzyme activities (Leinweber et al., 2008), and the composition of glomalin-related heat stable proteins in soil (Gillespie et al., 2011).

The use of Py-FIMS to investigate NaOH extracts of soil has been reported recently by Heumann et al. (2011). They could not find a link between the antioxidant capacities of a range of sandy soils and their net rates of nitrogen mineralization. However, the Py-FIMS results identified a group of sterol compounds that were negatively correlated with the net rates of N mineralization. They concluded that these sterols, although they were not antioxidants, were inhibiting the mineralization process. This finding was reinforced by Negassa et al. (2011) who found a negative correlation between the abundances of some sterols in agro-industrial by-products and enzyme activities of the N cycle following soil amendment with these by-products.

It is known, that acidified alkaline extracts (AAEs) of soils, such as the NaOH extracts used by Rimmer and Abbott (2011), resemble the FA fraction. This fraction is mainly comprised of polysaccharides, lignin phenolic and N-containing compounds, but lipids and peptides also contribute in varying proportions to the molecular–chemical composition (Schnitzer and Schulten, 1992; Schulten and Leinweber, 1993; Yabuta et al., 2008; Baigorri et al., 2009). Total humic extracts of organic wastes from plant residues in different composting stages were investigated by Py-GC/MS to follow the processes of SOM formation (Fuentes et al., 2010). However, none of these approaches have been used to explain the source of antioxidant capacity in soils. For plant materials it is known, that besides phenolic compounds, some amino acids and other nitrogen containing compounds contribute to the AOC (Suetsuna and Chen, 2002; Cano et al., 2003; Atmaca, 2004; Elias et al., 2008; Nimalaratne et al., 2011).

Because the quantification of a small number of specific compounds in soil extracts only accounted for a very small percentage of the AOC (Rimmer and Abbott, 2011), the aim of the present study was to obtain a broad characterization of the organic compounds present in AAEs of both vegetation and the corresponding soils using Py-FIMS. The information obtained in this way would allow us to test the hypothesis that certain organic compounds in the extracts, which potentially exhibit antioxidant properties, are significantly correlated to the AOC. We also hypothesised that these potential antioxidant compounds are similar in both vegetation and soil extracts.

All previous measurements of the AOC of soil extracts have been by the TEAC (Trolox equivalent antioxidant capacity) method (Re et al., 1999). In order to compare TEAC data to another AOC method, we have also used a PCL (photochemiluminescence) method (Popov and Lewin, 1996; Besco et al., 2007) in this study. We hypothesised that the data from the two methods show similar trends, if not absolute agreement.

2. Materials and methods

2.1. Soils and vegetation

Samples were collected from six of the sites previously used by Rimmer and Abbott (2011). They were chosen to represent a variety of soil and vegetation types with a range of antioxidant capacities. The location and other details of the vegetation and soils at each site are given in Tables 1 and 2. A single composite sample of aboveground vegetation and of soil (sampled at 5–10 cm depth) was collected at each site and put in glass bottles in the field to avoid any contamination that might occur from contact with plastic bags. The samples were stored at 4 °C until extraction, which was carried out after 2 days for soil samples and after 5 days for the vegetation samples.

2.2. NaOH extraction

The extraction procedure with 4 M NaOH followed that of Martens (2002), except that we doubled the quantities of soil (4 g) and vegetation (100 mg) and the volume of NaOH (10 ml). The extraction was carried out under standard atmospheric conditions (without any coating gas) in Teflon centrifuge tubes, which were autoclaved for 15 min at 121 °C and 100 kPa. The cooled samples were centrifuged and the supernatant solutions decanted and stored. The residues were rinsed with water (10 ml), centrifuged again, and the supernatant solutions added to the previous solutions. For each sample 10 ml of the combined solutions was acidified to pH < 2 with 4 M HCl, diluted to 25 ml with water, and finally centrifuged to remove any precipitate. Subsamples of the extract were freeze-dried for Py-FIMS. Either the solutions or the lyophilisates were analysed for total element concentrations (Table 3).

2.3. Measurements of AOC

2.3.1. Spectrophotometric analysis – Trolox equivalent antioxidant capacity (TEAC)

The method (Re et al., 1999) is based on the generation of the stable coloured free radical ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate), Fluka Chemicals, Steinheim, Germany) in aqueous solution. We used a UV–VIS spectrometer (Unicam model PU8625, Cambridge, UK) and measured at 734 nm. The ABTS was dissolved in deionised water to give a stock solution with a concentration of 7 mM. The radical cation (ABTS$^{\bullet+}$) was produced by reacting 9 ml of ABTS stock solution with 1 ml of 24.5 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature overnight before use. The ABTS$^{\bullet+}$ solution was diluted with deionised water (approximately 50-fold) to give an absorbance close to 0.70 at 734 nm.

Table 1
Soil and vegetation samples used in the study.

Site	Location	Soil series	Soil type	Horizon	Land use	Vegetation	Geology
1	55°13′N, 01°41′W	Rivington	Brown	A	Pasture	Permanent grass	Sandstone
2	55°13′N, 01°41′W	Hallsworth	Gley	A	Pasture	Permanent grass	Clayey glacial till
3	55°26′N, 01°52′W	Delamere	Podzol	Ah	Semi-natural	Bracken	Sandstone
4	55°26′N, 01°52′W	Newport	Brown	A	Pasture	Permanent grass	Glacial sands & gravel
5	55°07′N, 02°01′W	Crwbin	Lithomorphic	Ah	Pasture	Permanent grass	Limestone
6	55°08′N, 01°59′W	Fladbury	Gley	Apg	Semi-natural	Grass & sedge	Clayey river alluvium