Phenolic compounds in NaOH extracts of UK soils and their contribution to antioxidant capacity

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Abstract
Antioxidants are released during the extraction of soils with NaOH, which also releases phenolic compounds from plant and soil material. This raises the possibility that phenolics are important contributors to the antioxidant capacity (AOC) of soils. Both the AOCs and the concentrations of 12 phenolic compounds were measured in NaOH extracts of a range of UK soils. Samples of surface and subsurface horizons were taken from 24 sites representing the major soil types (brown soils, gleys, podzols, peats and lithomorphic soils). The internal standards method was used to quantify the phenolics, which were detected by gas chromatography. The AOC of the extracts was measured using the ABTS free radical method. There were differences in the phenolic distributions extracted from soils with different land uses/plant inputs, as well as differences between surface and subsurface samples. A linear relationship was found between the AOCs of the extracts and the sum of the phenolic compounds. The AOCs of the individual phenolic compounds were also measured. The calculated contribution of the individual phenolics to the AOC of the extracts was small and less than 10% of the total AOC in all cases. Thus the measured phenolic compounds were not important contributors to the AOCs, and other unidentified antioxidant compounds were probably present.

Key Words
Soil organic matter mineralization

Introduction
Phenolic compounds in soils have been studied for at least 50 years and much of the work related to podzolization processes (Bloomfield, 1952; Vance et al., 1986). The relationship between phenolics in crop residues and soil processes, such as residue decomposition, nutrient release, and aggregate formation, was investigated by Martens (2002a, b), who established that phenolics were metabolized in soil at a much slower rate than the carbohydrates and amino acids.

Many naturally occurring antioxidants are phenolics, for example vitamin E. Antioxidant molecules terminate the chain reaction of damaging free radical formation by being transformed into unreactive, stable free radicals and thereby control oxidation processes (Halliwell and Gutteridge, 2007). The degradation of food involves oxidation by free radicals and is retarded by antioxidants (Gaman and Sherrington, 1990). Therefore, it was hypothesised that antioxidants are present in soil and that they are involved in the protection of soil organic matter from oxidation (Rimmer, 2006). Subsequently we showed that antioxidants can be extracted from soils with NaOH, in proportion to their organic carbon contents (Rimmer and Smith 2009).

Because many phenolic compounds have antioxidant properties, and because previous studies have demonstrated the presence, and importance, of phenols in soils (e.g. Martens, 2002a, b), the objectives of the present study were to: measure the antioxidant capacity (AOC) and the concentration of a group of phenolic compounds in NaOH extracts of soils, and to calculate the contribution of the measured phenolics to the AOC. By using samples of surface and subsurface horizons from a wide range of UK soil types from sites with a range of land uses, we aimed to identify any differences in both the phenolic compounds and the AOC resulting from differences in horizon, soil type, and land use.

Materials and methods

Soils
Samples from 24 sites, representing the major soil groups in the UK, were collected. By separately sampling the different soil horizons at each site, we obtained samples from 48 horizons for the study. The sites were selected to cover the soil groups: brown soils, gleys, podzols, peats, and lithomorphic soils (Avery, 1990).
**Measurement of antioxidant capacity**

The method chosen was the Trolox equivalent antioxidant capacity (Re et al., 1999). This uses a stable coloured free radical in aqueous solution, with the measurement of antioxidant capacity being the decrease in absorbance of the solution in a UV-VIS spectrophotometer following addition of the antioxidant. The radical used is 2,2’-azinobis(3-ethylbenzothiazoline-6-sulphonate) (ABTS). Calibration with a standard antioxidant, Trolox (an analogue of vitamin E), allowed the absorbance decrease results to be expressed as Trolox equivalent concentrations (µM).

**Extraction method**

Two extraction methods were used in the study of phenolic acids by Martens (2002a), namely 1 M NaOH at room temperature and 4 M NaOH autoclaved at 120°C. Previously we used 1 M NaOH to bring antioxidants into solution (Rimmer and Smith, 2009). Here we compared 1 M and 4 M NaOH methods and found that 4 M NaOH was significantly more effective in bringing antioxidants into solution, and was adopted.

**Analysis of phenolic compounds**

The 12 phenolics quantified were: p-hydroxy-benzaldehyde, vanillin, p-hydroxy-acetophenone, acetovanillone, p-hydroxy-benzoic acid, vanillic acid, syringaldehyde, p-coumaric acid, acetylsyringone, syringic acid, ferulic acid, and sinapic acid. These are the same as those quantified by Martens (2002a), and are the most abundant ones in the extracts, although it is likely that many others, such as guaiacol, syringol and their derivatives will also be present. The isolation and analysis of these compounds was based on the procedures described by Martens (2002a). The samples were analysed on a gas chromatograph and compounds were identified by comparison of retention time with those of standards. Compounds were quantified by using the internal standards method.

**Antioxidant capacity of phenolic compounds**

The antioxidant capacity of each of the 12 phenolic compounds was measured by dissolving 10 g of the compound in ethanol and diluting with deionised water. Aliquots of these solutions were assayed for antioxidant capacity using ABTS with calibration against Trolox, as described above (Re et al., 1999).

**Results and discussion**

**Phenolic compounds**

The concentrations of the extracted phenolic compounds are shown in Figure 1. The overall pattern (Figure 1a) shows that the three compounds with the greatest concentrations were: p-hydroxybenzoic acid, vanillic acid, and ferulic acid. These compounds were also dominant for the subset of surface samples, which had a similar pattern of distribution (data not shown). By contrast for the subsurface samples, vanillin had the greatest median concentration, followed by vanillic acid and p-hydroxybenzoic acid. Martens (2002b) in his study of soil amended with plant residues found that there was only an increase in the concentration of p-hydroxybenzoic acid over the 84-day incubation. This increase was attributed to its production by microbial synthesis (Moorman et al., 1992), which may explain why overall it had the greatest concentration in the soils of this study, and particularly for surface samples.

The effect of land use on the concentrations of phenolic compounds extracted from surface soils is also shown in Figure 1. Pasture was the dominant land use and the pattern seen (Figure 1b) was similar to that for all samples (Figure 1a). In arable soils (Figure 1c) there was a completely different pattern with acetylsyringone having the greatest concentration, while vanillic acid was most important in both semi-natural and woodland soils (data not shown).

Martens (2002a) presented data on phenolic compounds from surface soils of a single soil type but under different management/cropping. The methods of extraction, purification and quantification were the same as in the present study. The concentrations obtained were of a similar magnitude, and the dominant compounds were similar to those above. In the prairie soil these were: p-coumaric acid, ferulic acid, and p-hydroxybenzoic acid. For the cropped soils, acetylsyringone replaced ferulic acid in the list, as it did in the arable soils of the present study. We conclude therefore that the vegetation, and particularly vegetation residues, play an important role in determining the phenolic compounds extracted.

The concentrations of the phenolic compounds measured in the extracts were summed. The median value of the summed amounts of the phenolic compounds for surface soils was approximately 10 times greater than
that for subsurface horizons, and reflected differences in the organic matter content of the samples. This is in agreement with Martens (2002b), who reported that the total phenolic content of a single soil incubated with various plant residues increased as organic C increased.

Figure 1. Box plots showing the concentration of the 12 phenolic compounds extracted from (a) all samples, and from surface samples of (b) pasture and (c) arable soils. The line within the box marks the median concentration and the boundaries of the box indicate the 25th and 75th percentiles. Whiskers indicate the minimum and maximum concentrations, excluding outliers.

Antioxidant capacity
The median values of antioxidant capacity ranged from 1.32 to 102 μmol Trolox equivalent g⁻¹ soil, and there was a positive correlation between AOC and C content ($r^2 = 0.814, P < 0.001$). Surface horizons had greater antioxidant capacities than subsurface horizons and the difference was significant ($P < 0.001$). This increase in antioxidant capacity with C content agrees with earlier findings (Rimmer and Smith, 2009).
There were significant differences between soil types with brown soils and gleys, which had small carbon contents, having the smallest antioxidant capacities and peats the largest, with the other soil types being intermediate. There were clear differences with land use; the order of increasing antioxidant capacities was arable < woodland < pasture < semi-natural vegetation, which again reflected increasing carbon contents.

The contribution of individual antioxidant compounds to the measured antioxidant capacities
The sum of phenolic compounds was positively correlated with the antioxidant capacities ($r^2 = 0.767, P < 0.001$). Six of the compounds had measurable antioxidant capacities (vanillin, vanillic acid, p-coumaric acid, syringic acid, ferulic acid, and sinapic acid). The sum of those six phenolics was also positively correlated with the antioxidant capacities ($r^2 = 0.817, P < 0.001$). This suggested that these antioxidant compounds could be important contributors to the overall antioxidant capacity.

This was tested by using the measured antioxidant capacities of the six antioxidants and their concentrations to calculate their contribution to the total antioxidant capacities of the extracts. The results showed that the contributions were very small. In surface samples it was generally greater (median value: 3.07%) than in subsurface samples (median value: 1.31%). A possible explanation for this is that the overall antioxidant capacity of the six compounds acting together is greater than the sum of their individual capacities or, more likely, that there are other unidentified antioxidant compounds present in the extracts.

Conclusions
Increasing concentrations of phenolic compounds were extracted from different soils with increasing organic matter content. Large differences were found between the amounts of individual phenolic compounds extracted from different soils which were dependent on the land use. This effect will be the result of different plant residues with different phenolic compositions being incorporated in the soil. Antioxidant capacities were also proportional to the SOM content, and this explained differences between soil types and land uses.

We hypothesised that phenolic compounds would be important contributors to the antioxidant capacity of soil extracts. We found that the sum of the phenolic compounds measured was positively correlated with the antioxidant capacities (AOC), which agreed with the hypothesis. However, not all of the phenolic compounds that we quantified had antioxidant capacity, and those that had were only minor contributors (< 10%) to the overall AOC of the extracts. This suggests that other unidentified antioxidant molecules were probably present in the extracts.

References