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Temperature and duration of extraction affect the biochemical composition of soil water-extractable organic matter

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ABSTRACT

Water-extractable organic matter is regarded as readily available substrate for soil microbes. However, little is known about the influence of extraction temperature and duration on its biochemical composition. The effect of temperature (20 *vs.* 80 °C) and extraction duration (1–24 h) on the amounts of water-extractable organic C (WEOC), water-extractable organic N (WEON), carbohydrates, phenolics, ninhydrin-reactive organic N (NRON), and mineral N were evaluated using four agricultural soils from New Zealand and eastern Canada. More WEOC and WEON were extracted in hot than in cold water, and the same was found for all biochemical compounds tested. Biochemical components increased rapidly during the first 4 h of extraction at 80 °C, and at a slower rate thereafter. In contrast, at 20 °C, concentrations of all measured components were at or close to their maximum after 1 h and showed little change thereafter. Glucose and nitrate in the 20 °C extracts both declined substantially between 1 and 24 h, likely due to microbial activity. Moreover, the disappearance of glucose induced a decline in the ratio of carbohydrate-C to phenolic-C. The carbohydrates measured in water extracts at both temperatures were predominantly of microbial origin after 1 h. However, the proportion of microbial carbohydrates gradually declined and the proportion of plant carbohydrates increased from 1 to 24 h at 80 °C. Based on these findings, we recommend limiting extractions to 1 h or less at 20 °C and to 4 h or less at 80 °C to minimize compositional changes that may occur during longer extraction periods.

1. Introduction

Water-extractable organic matter (WEOM) is a source of readily available C and nutrients for soil microbes (Burford and Bremner, 1975; McGill et al., 1986; Gregorich et al., 2003; Marschner and Kalbitz, 2003). Although it generally comprises <2% of total soil organic matter (SOM), its high turnover rate and solubility means that it has a key role in many chemical and biological processes in both topsoil and subsoil (Kalbitz et al., 2000; Murphy et al., 2000; Peterson et al., 2013). It has also been shown to facilitate the transport of metals, nutrients, pesticides, and hydrophobic contaminants (Tipping et al., 2006; Zhang and Zhang, 2010; Bolan et al., 2011). These functions are thought to involve different molecular components of WEOM (Kalbitz et al., 2003; van Hees et al., 2005), including carbohydrates, phenols, amino acids, and organic acids (Haynes, 2000; Jones et al., 2004; van Hees et al., 2005). The biodegradability of dissolved organic matter tends to increase as its content in carbohydrates, low-molecular weight organic acids and amino acids increases, whereas aromatic and hydrophobic compounds are more recalcitrant (Marschner and Kalbitz, 2003).

The temperature of extraction can influence the composition and biodegradability of WEOM. It was found that hot-WEOM (extracted at 80 °C) was enriched in carbohydrates (Haynes and Francis, 1993) and that a greater proportion of WEOM was biodegradable when extracted at 80 °C than at room temperature (Gregorich et al., 2003). There is no standard protocol for determining the quantity and quality of WEOM in soils. A wide range of extraction temperatures (e.g. 20–100 °C) and extraction times (e.g. 1–24 h) have been used (Zsolnay, 1996; Chantigny, 2003). Previous research has shown that water-extractable C can increase substantially as extraction temperature increases (Birch, 1959; Gregorich et al., 2003; Martinez et al., 2003; Curtin et al., 2006; Chantigny et al., 2010), but the influence of extraction conditions on the biochemical composition of WEOM is essentially unknown. Our objective was to quantify the influence of extraction temperature and duration, and their interaction, on biochemical

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composition of organic matter extracted in water from agricultural soils.

2. Materials and methods

2.1. Selection of soils

Four loamy soils representing different paedogenetic development conditions and management histories were used in the study. Two soils (Udic Dystocrept) were collected from a field trial in Lincoln, New Zealand (43°40′ S; 172°28′ E; mean annual temperature (MAT), 11.4 °C; mean annual precipitation (MAP), 680 mm). One of the soils was under long-term, sheep-grazed grassland (perennial ryegrass/white clover, *Lolium perenne* L./*Trifolium repens* L.) and the other was under conventionally cultivated (ploughed to 20 cm) arable cropping for 8 yr [rotation of barley (*Hordeum vulgarum* L.) – wheat (*Triticum aestivum* L.) – pea (*Pisum sativa* L.) – barley – pea – barley] and received standard rates of mineral fertilisers.

Two other soils (Typic Humaquept) were collected from long-term experimental plots (46°05′ N; 71°02′ W; MAT, 4.2 °C; MAP, 1213 mm) in eastern Canada. One of the soils was under continuous corn (*Zea mays* L.) for 28 yr, was ploughed each fall (25 cm depth), and received mineral fertilisers each spring. The other soil was under heavily manured (100 m^3 pig slurry ha^{-1} yr^{-1}) grassland [mixture of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.)]. The grassland site had been ploughed and re-seeded every 7 yr for 25 yr.

The New Zealand and Canadian soils had similar pH (5.6–6.2) and texture (loam to silt loam). The grassland soils had more total organic C (31.1–32.1 g kg^{-1}) and total N (2.3–2.6 g kg^{-1}) than their arable counterparts (19.8–22.6 g C kg^{-1}; 1.4–1.9 g N kg^{-1}). The C to N ratio was lower in New Zealand soils (11.9–12.3) than in Canadian soils (13.5–14.1). More details about characteristics of these soils are found in Chantigny et al. (2010).

2.2. Soil collection and handling

All soils were sampled by collecting the top 15 cm with a 0.1-m diameter stainless steel corer. For grassland soils, the surface thatch was removed and the top 15 cm of the A horizon was sampled. Fifteen cores were collected, mixed to make one composite sample (*ca.* 4 kg of soil), and sieved to 4 mm. All identifiable plant residues and roots were removed by hand. The sieved soil samples were air dried for 2 wk at room temperature (18–20 °C) with daily hand mixing to ensure homogeneous drying of the soil.

2.3. Water extractions and analyses

All extractions were carried out in triplicates. Thirty-gram samples of the air-dried soils were placed in 250-mL centrifuge bottles and shaken (end over end) for 5 min with 60 mL of deionised water. The bottles were then placed in an incubator at 20 °C, or in a water bath at 80 °C, and incubated without agitation for 1, 4, 8, 12 or 24 h. After the incubation, samples were hand-shaken for ~10 s to re-suspend the soil and then centrifuged at 7000 × *g* for 10 min. The supernatant was decanted and passed through a glass fibre filter paper (Whatman #934-AH). The filtrates were stored at 1 °C until analysed.

Nitrate-N, NH_4–N and total dissolved N (TDN) were measured within 72 h of extraction using an automated flow injection analyser fitted with an online UV-catalysed persulphate oxidation unit (Model QuickChem 8000 FIA+, Lachat Instruments, Loveland, CO). The water-extractable organic N (WEON) was taken as the difference between TDN and mineral N (NO_3–N + NH_4–N). The concentration of C dissolved in the water extracts was determined within 72 h using an automated total C analyser (Model TOC-5050, Shimadzu Corp., Kyoto, Japan). Total and inorganic C were measured and water-extractable organic C (WEOC) was taken as the difference.

The concentrations of organic components in the soil extracts were measured within 4 d using colourimetric methods as described in Chantigny et al. (2008). Total hexoses and pentoses were determined using the anthrone–sulphuric acid (glucose as standard) and the orcinol-ferric chloride hydrochloric acid (xylose as standard) methods, respectively. Phenolics were determined using the Folin–Ciocalteu reagent (vanillic acid as standard). Total ninhydrin-reactive N was determined (Joergensen and Brookes, 1990) and ninhydrin-reactive organic N (NRON) was estimated by subtracting NH_4–N from total ninhydrin-reactive N. Neutral carbohydrates were determined in the water extracts by acid hydrolysis as described in Chantigny et al. (2000), and quantification of arabinose, galactose, glucose, mannose and xylose by high-performance anion-exchange chromatography coupled with pulsed amperometry (Chantigny and Angers, 2008). The ratio of microbe- to plant-derived carbohydrates was calculated as the ratio of galactose + mannose to arabinose + xylose (Murayama, 1984; Chantigny et al., 2000).

2.4. Data analysis

The effects of extraction temperature (T), duration (D) and their interaction ($T \times D$) on water-extractable C and N were tested using a mixed effects model in the ASReml (Gilmour et al., 2009) package for R (R Development Core Team, 2011). A log – log model was fitted which is a linearization of the model $y = ax^b$ where y is the response variable, in this case the various water-extractable C and N compounds, and x is the explanatory variable (temperature, extraction time, and the interaction). The coefficient b in the model controls the shape of the curve, where positive and negative values reflect an increase or decrease in concentration, respectively. The soil types were included in the model as a random effect. The graphs in Figs. 1–3 show the data points along with fitted curves (e.g. compound concentration as a function of extraction time) for each temperature separately.

3. Results

3.1. Amounts of water-extractable C and N

Given the large number of parameters tested in the present study, results on the statistical effects of temperature and duration of extraction are presented for all parameters in Table 1, whereas only the most relevant results are presented in details in Figs. 1–3. The concentrations of WEOC, WEON, and all biochemical components were greater at 80 than at 20 °C (Table 1). At 20 °C, the concentrations of most measured parameters were already close to their maximum after 1 h of incubation and generally showed limited changes from 1 to 24 h (Table 1; Figs. 1 and 2). The only exceptions were glucose-C (Fig. 1e) and NO_3–N (Fig. 2e), which significantly declined over time (Table 1). As glucose accounted for more than 50% of soil hexoses (Fig. 1), its gradual decline at 20 °C reflected in the concentration of hexoses-C (Fig. 1c) and total carbohydrate-C (Table 1).

At 80 °C, the concentration of all measured constituents increased over time (Table 1, Figs. 1 and 2), except for NO_3–N which remained constant (Fig. 2f). The NO_3–N levels were similar to values measured after 1 h at 20 °C (Fig. 2e). The concentrations of biochemical components increased rapidly