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Enzyme activities and compositional shifts in the community structure of bacterial groups in English wetland soils associated with preservation of organic remains in archaeological sites

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ABSTRACT

To extend our previous research on preservation *in situ* of organic archaeological remains in wetland soils, physicochemical variables and extracellular enzyme activities (β-D-glucosidase, phosphatase, and leucine aminopeptidase) were measured in two archaeological wetland soils in northern England, from the soil surface down to 100 cm during two years. These two locations, with a proven potential to contain and preserve archaeo-environmental remains, were of interest because they had different hydrological and land-management history. In addition, for one of the sampling years, the structure of soil bacterial communities was monitored using polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE) of 16S rRNA gene-coding fragments amplified from soil DNA, using primers specific for eu bacteria, actinomycetes, and α-proteobacteria. The physicochemical monitoring data indicated that at Sutton Common the buried conditions were less stable and more heterogeneous throughout the soil profile than at Hatfield Moor. Extracellular enzyme activities decreased with depth at both sites and higher average enzyme activity was found at Hatfield Moor than at Sutton Common. Phosphatase and leucine aminopeptidase were the most active enzymes throughout the soil profile. Our results indicated that the enzymatic potential for organic matter (OM) degradation at both sites was strongly correlated to pH and OM content in the soil. DGGE patterns for eubacterial, α-proteobacteria, and actinomycete populations indicated intra-site changes in community structure with time of sampling and depth. The sampling site with a high and stable water table, Hatfield Moor, showed higher enzyme activities above 50 cm depth and as a consequence had more potential for OM degradation than the site with fluctuating hydrological conditions. These trends provide insight into the potential for biodegradation over time and with depth at these two sites, information that is relevant to the *in situ* preservation of buried organic archaeological artefacts in wetland soils.

1. Introduction

Knowledge of microbial community composition and function is critical for an understanding of soil organic matter (OM) dynamics, including the degradation or preservation *in situ* of buried organic archaeological artefacts. The ongoing discovery of new archaeological sites in wetlands generally coincides with limited funds for excavation and with lack of space in museums for the adequate preservation, conservation, and storage of artefacts. As a result, the European Convention on the Protection of the Archaeological Heritage (Council of Europe, 1992) states that *in situ* preservation, where possible, is the preferred option to protect and conserve archaeological remains (Gregory et al., 2008). Therefore, to improve management and preservation at archaeological sites, a better knowledge of the burial environment in which archaeological remains are preserved is needed (Huisman et al., 2008).

In this study, to improve the understanding of the factors affecting OM degradation in the burial environment, we monitor several environmental variables (pH, temperature, moisture content, etc.) and their influence on hydrolytic enzyme activities over a period of two years through the soil profile. We consider that monitoring through the soil profile for the presence and activity of

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microorganisms is a fundamental tool to assess the potential for biodegradation *in situ* at archaeological sites.

We studied soil extracellular enzyme activities, as these are primarily responsible for OM decomposition (Kang and Freeman, 1998; Nannipieri et al., 2002). Extracellular enzymes play an important role within soil ecology as they are involved in the cycling of nutrients, and they act as indicators of physicochemical change in soil (Burns, 1982). Microbial enzymatic activity is very sensitive to fluctuation in external factors (Bandick and Dick, 1999) and is an inexpensive and easy technique to employ; for this reason enzymatic assays have been widely used to study the effect of environmental change in soil (e.g., Acosta-Martinez et al., 2003; Trasar-Cepeda et al., 2008; Kolehmainen et al., 2009). Among the most valuable enzymes for studying the potential for OM decomposition in soil are glucosidases, phosphatases, and aminopeptidases. Glucosidases break down polysaccharides; β-D-glucosidase contributes to the degradation of cellulose and has been widely used as an indicator of soil quality under different management regimes (Gil-Sostres et al., 2005). Phosphatase is vital in the mineralization of phosphate from organic sources (Sinsabaugh et al., 2008) and is considered to be a good indicator of the quality and quantity of OM in soil (Bergstrom et al., 2000). The enzyme aminopeptidase is involved in the degradation of proteins and subsequent mineralization of nitrogen in soils (Tabatabai et al., 2002); it has also been used as an indicator of OM decomposition (Sinsabaugh et al., 2008).

The potential for enzyme synthesis is determined by the abundance and composition of the microbial community present in soil. As a consequence, changes in microbial communities associated with environmental factors should be reflected by changes in enzyme activity (Kandeler et al., 1996). It has been previously shown that bacteria are the principal agents of organic matter decomposition in these environments (Björdal et al., 2000; Powell et al., 2001; Gregory et al., 2002; Helms et al., 2004). For this reason, in this study, temporal and spatial variation of soil bacterial community structure was evaluated using a molecular fingerprint technique (PCR-DGGE). Culture-dependent techniques recover less than 1% of the total bacterial community present in soil samples (Torsvik et al., 1998; Nannipieri et al., 2003). The use of molecular techniques allows for the characterization of those microorganisms that currently cannot be cultured, given a more accurate representation of the microbial community within the soil profile (Borneman et al., 1996; Felske et al., 2000). The use of eubacterial primers for DGGE shows only the most abundant species in the soil samples (Boon et al., 2002). In order to assess compositional shifts in the community structure of specific bacterial groups that might play an important role in the biodegradation process, we used specific PCR primers for two different phylogenetic groups: α-proteobacteria and actinomycetes.

The current study aimed to extend the authors' previous research on preservation *in situ* of organic archaeological remains in wetland soils. With that purpose, we assessed the influence of changes in the burial environment upon hydrolytic enzyme activity and microbial community composition down the soil profile and over time at two archaeological wetlands. The results will assist heritage managers to evaluate the potential for in situ preservation of organic artefacts in archaeological sites.

2. Materials and methods

2.1. Site description

The first sampling site, Hatfield Moor (53°38′18″ N, 00°53′51″ W) forms part of the Humberhead Peatlands National Nature Reserve and has been classified as a site of special scientific interest (SSSI) and as a special protection area under EC legislation. The environmental importance of Hatfield Moor is due to the presence of a high percentage of raised bog that, although degraded, is still thought to be capable of natural regeneration. At Hatfield Moor a high water table has been kept by the (then) Countryside Agency's "Value in Wetness" initiative, as a new approach to improve land use and water resources management in the area (Pasley, 2007). Archaeological finds in the area include two wooden trackways; the first is from the Late Neolithic (Chapman and Geary, 2006) and the second one is dated to the Bronze Age (Buckland, 1979). In addition, a series of prehistoric finds (e.g., Neolithic flint flakes, and Mesolithic and Neolithic axes), lithic scatters, and worked timbers have also been found in the area (Dinnin, 1997; Van de Noort and Fenwick, 1997).

The second site studied was at Sutton Common (53°37′0″N, 1° 9′0″W), where important Iron Age archaeological features and organic remains, mainly wood and other organic material, have been found, below the depth of ploughing (Parker Pearson and Sydes, 1995). Sutton Common contains a "scheduled ancient monument" (SAM) and an SSSI. Although of archaeological relevance, the area has been drained and is currently used as pasture for cattle (Cheetham, 2004).

At both sites wood (mainly oak) compacted in an organic matrix was frequently found through the soil profile, particularly below a depth of 30 cm. The subsequent analysis that was carried out included this organic matrix. The presence of these wooden remains within the soil were useful to illustrate the potential degradation of organic materials, specifically how it might affect organic archaeological remains.

These two sites were chosen in order to evaluate the effects of both land-management regime and different hydrological status upon soil microbial enzyme activity and bacterial community structure over time and throughout the soil profile.

2.2. Soil physicochemical characterization and sampling procedure

The monitoring methods and experimental setup are given in detail in Douterelo et al. (2009, 2010). To summarize, monitoring and sample collection were carried out at two-month intervals for a period of two years from April 2004 to April 2006 at the two sites. Soil samples were analysed for a number of physicochemical characteristics (organic matter, moisture content, pH, and temperature) at five depth intervals (0–10 cm, 30–40 cm, 50–60 cm, 70–80 cm, and 100–110 cm). Soil samples were recovered using a (Eijkelkamp) hand auger, which collected approximately 450 cm^3 of soil, from which sub-samples of 100–200 g wet weight were taken. Samples were stored in the dark at 4 °C until enzyme activity assays were undertaken on the following day; consequently conditions largely resembled the soil environment (Trasar-Cepeda et al., 2000; Goberna et al., 2006; Wallestein et al., 2009). Samples for molecular genomic work were stored at −20 °C for subsequent analysis.

2.3. Extracellular enzyme activity

Enzyme activities were estimated using fluorogenic 4-methylumbelliferone substrates for phosphatase and β-D-glucosidase (Sigma–Aldrich, UK) and 7-amido-4-methylcoumarin (Sigma–Aldrich, UK) for leucine aminopeptidase. For each enzyme assay, three replicate 1 g wet wt l^{-1} soil suspensions were used, while boiled and cooled soil suspension blanks were also used. Fluorogenic substrate was added to suspensions to give a final concentration of 200 $\mu mol\ l^{-1}$ and the suspensions were then incubated in darkness for 5 h at 10 °C. After incubation, the soil suspensions were centrifuged at 2225 RCF for 5 min and a 5 ml aliquot was taken and added to 0.4 ml of pH 10 borate buffer solution (BDH, UK). The fluorescence intensity was measured using a fluorometer (Turner Designs Model 10, Steptec Instrument Services, Bedforshire, UK), which was fitted