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Temporal and land use effects on soil bacterial community structure of the machair, an EU Habitats Directive Annex I low-input agricultural system

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ABSTRACT

The machair, a low-input agricultural system in the extreme north-west of Scotland, is a rare example of an extant system that has never been intensively cultivated. The bacterial community structure represents an opportunity to test variation connected with temporal, soil compartment and land use factors. To achieve this objective a two-year, three season sampling regime over the three major land uses present: cropped, fallow and grasslands was performed. Bacterial communities of rhizosphere and bulk soil compartments were assessed using terminal restriction fragment length polymorphism (T-RFLP). Machair bacterial community structure was primarily determined by soil compartment and temporal factors, with differences both between and within years highly significant. Although land use was not the main determinant of bacterial community, clear differences were detected. Cropped and fallow sites contained a similar bacterial community while grassland sites were different. Correlation with soil physico-chemical factors indicated that machair bacterial community structure may be driven to some degree by soil moisture content.

This study highlights the need to take seasonal and annual variation into account when assessing bacterial communities in an agricultural setting.

1. Introduction

The importance of soil microbial communities in ecosystem functioning is unequivocal. Vital ecosystem functions such as nutrient cycling and decomposition in both natural and managed ecosystems are performed by bacteria and support a wide range of ecosystem services (Dominati et al., 2010). However, our understanding of the factors that structure and regulate these communities across a variety of ecosystems is poor (Fierer et al., 2009). On a global scale, bacterial community composition, biomass and distribution are believed to be primarily related to physico-chemical factors such as pH (Fierer and Jackson, 2006), soil type (Bossio et al., 1998) and soil organic matter (Wardle, 1992), while at a local scale both abiotic and biotic factors, such as vegetation composition (Eisenhauer et al., 2010; Nunan et al., 2005) and grazing (Attard et al., 2008; Bardgett et al., 2001), are known to contribute towards determining bacterial community dynamics.

Temporal variation in bacterial community structure can be large (Bossio et al., 1998) and variable (Griffiths et al., 2003), although the effect of inter-annual variation in stable ecosystems seems to be largely unexplored as the majority of studies focus on single years.

Bacterial diversity and structure can differ considerably between arable systems and natural habitats due to management practices such as fertiliser, herbicide and irrigation application (Jangid et al., 2008; Steenwerth et al., 2003). Microbial diversity and community structure differ at different disturbance intensities (Allison et al., 2005; Coleman et al., 1983; Wu et al., 2008) but on the whole, microbial communities are thought to be predominantly affected by changes in edaphic factors (Kuramae et al., 2012; Lauber et al., 2008; Schutter et al., 2001). For example, application of soil amendments and fertilisers can result in shifts in bacterial community structure (Pérez-Piqueres et al., 2006; Seghers et al., 2003) and increases in both microbial biomass and activity (Albiach et al., 2000; Debosz et al., 2002; Enwall et al., 2007; Ruppel et al., 2007).

Organic and low input agro-ecosystems are less reliant on external inputs than intensive agricultural systems and are known to have a higher microbial biomass (Mäder et al., 2002) and diversity (Jangid et al., 2008). Agricultural practices which rely heavily on large amounts of external inputs are increasingly viewed as unsustainable and there has been a growing interest in sustainable farming systems (Kristiansen and Merfield, 2006; Tilman et al.,

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2002) and the ecosystem goods and services that they offer (van der Putten et al., 2004). The pivotal role that bacteria play in providing some of these essential ecosystem services (Dominati et al., 2010) means a better understanding of the complex set of factors that affect soil microbial dynamics is required in order to adequately manage soil systems (van der Putten et al., 2004).

In Europe, areas under traditional cropping are increasingly under threat with numerous protection measures in place to protect these systems (Bignal and McCracken, 1996). Machair is an ecosystem found predominantly on calcareous soils with high shell content in coastal areas of the north-west of Scotland and Ireland. It has a long history of traditional land management (crofting) comprising low-input, rotational arable areas interspersed with semi-natural grasslands. The grassland areas are permanent and do not form part of the managed rotation, and typically represent areas of abandonment from cultivation, as they are often distant from the croft or difficult to manage. Arable areas are fertilised with a combination of mineral fertiliser and rotted seaweed prior to shallow ploughing and cropped with farm-saved seed. Although the nature and extent varies per location, this traditional land management is an essential part of the machair system and both intensification and abandonment threaten its future (Crawford, 1997). As one of the rarest ecosystems in Europe with an estimated area of approximately 25,000 ha (Anon., 1999), machair is listed on the EU Habitats Directive Annex I, with large parts designated as UK Sites of Special Scientific Interest, Special Areas of Conservation and Special Protection Areas.

To our knowledge no research has been conducted on any aspect of soil bacterial community dynamics on the machair. The aim of this study was to determine the impact of temporal (annual and inter-annual) and land use (cropped, fallow and grassland) factors on soil bacterial community structure in this system. We also assessed a range of associated physico-chemical factors and determined if associations existed with bacterial community structure.

2. Materials and methods

2.1. Study site and sampling regime

In year 1, 10 locations were sampled in April/May, July and October 2007 along a north-south gradient, of approximately 60 km, across the islands of North Uist, Baleshare, Benbecula and South Uist in the Outer Hebrides off the west coast of Scotland (Table 1). At each location three fields corresponding to the three main land use types (cropped, fallow and grassland) within the region defined as "machair grassland" (Anon., 1999) were selected. Each field was randomly split into three, relatively equal replicate areas, yielding nine sampling points per location. At all locations, a typical machair arable rotation of two year cropped, two-year fallow was in place. To investigate year-to-year variation in bacterial community structure, sampling was repeated in year 2 (2008) on a subset of four locations (Table 1). Thirty year long term average values for the Outer Hebrides (Stornoway) are: total annual precipitation 1249 mm, with over 205 days of $\geq$1 mm, and with a mean air temperature of 8.1 °C (Met. Office, 2013).

Cropped sites comprised a mixture of mainly rye (*Secale cereale* L.) and small oats (*Avena strigosa* Schreb.) grown from locally produced seeds resulting, due to a high contribution from perennial non-crop plants, in a diverse arable plant community (Crawford, 1990). Fertilisation generally consisted of a mixture of mineral fertiliser and composted seaweed applied a short time prior to ploughing and seeding in spring. Fallow sites were previously cropped but left to recuperate for two years before being reused; no management, aside from grazing, took place in these areas during this time. Permanent grassland sites generally occurred in areas that were unsuitable for cropping; being either too rocky or wet and most were unfertilised, though a few locations received limited fertilisation to maintain productivity. As a consequence, these sites were not considered to be a true control for their cropped and fallow counterparts but were the only semi-natural machair sites available for comparison with arable areas. Grazing by cattle and/or sheep took place in all areas, but was restricted to the post-harvesting, pre-ploughing period in cropped areas.

In each replicate plot, two cores (Ø 6.5 cm × 10 cm) were taken for bacterial community structure analysis with each plot targeting both *Bellis perennis* L. and *Festuca rubra* L. The cores were randomly distributed within each plot and taken with each plant central to each core. A further composite soil sample was taken throughout each replicate plot for physico-chemical analysis combining 20 small cores taken from the top 10 cm using a grass plot sampler (Eijkelkamp, Giesbeek, Netherlands). This composite sample was taken at a single time point in year 1 (spring) and at all sampling times in the second year. *B. perennis* L. and *F. rubra* L. represented the two dominant broad functional plant types (forb and grass) found at the majority of sites and were selected to take any possible host effect into account (Wieland et al., 2001). If either of these two plant species did not occur in a particular replicate, a core containing roots and soil linked to a different plant species within the same functional group was sampled for all replicates in that field; *Potentilla anserina* L. or *Ranunculus repens* L. were substituted for *B. perennis* while *Agrostis stolonifera* L. or *A. strigosa* were substituted for *F. rubra*. It was rare that *B. perennis* and *F. rubra* were not present and an analysis demonstrated that plant identity had no significant effect on bacterial community structure (data not shown). All cores included intact plants; these were returned to the laboratory and stored under ambient conditions with light. Thereafter each core was separated into bulk and rhizosphere soil with the bulk soil defined as soil easily separated from the root zone. Upon core destruction sub samples (approximately 2 g) of each soil compartment was taken for DNA extraction and immediately frozen to −80 °C.

2.2. Physico-chemical analysis of soils

All physico-chemical measurements were conducted on composite soil samples collected in April/May of year 1 and May, July and October of year 2. This allowed for comparison between physico-chemical properties and bacterial community structure for land use in year 1, land use and season in year 2 and year in April/May of years 1 and 2.

Soil pH was measured approximately 20 min after thoroughly mixing 10 g of soil in 25 ml of 0.01 M $CaCl_2$. Soil moisture content (DW) was determined by the wet mass percentage method (Tan, 2005) using approximately 10 g of soil, with the soil dried at 60 °C for at least 24 h. For dissolved organic carbon (DOC), NO_3^--N and NH_4^+-N measurements, 10 g of soil was mixed continuously with 40 ml of 1 M KCl for 1 h, filtered on 125 mm filter paper (Whatman No. 1, Maidstone, UK) and run on a Skalar SANplus Segmented Flow analyser (Skalar Analytical B.V., Breda, The Netherlands) according to the protocol listed by the manufacturer. Phosphorus (P) was extracted from 2 g of soil using anionic exchange resin strips (Saggar et al., 1990) and the P content of each extract was measured using the malachite green method (Irving and McLaughlin, 1990).

2.3. Bacterial community structure

DNA was extracted from approximately 1 g of frozen soil using a phenol:chloroform extraction method in a 96 well format according to Deng et al. (2010). Samples were randomised prior to analysis to remove variability due to plate variation. DNA was amplified using the general bacterial primers 16F27-FAM