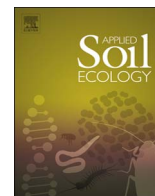




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Comparative effects of prolonged freshwater and saline flooding on nitrogen cycling in an agricultural soil

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ABSTRACT

Due to climate change, the frequency and duration of flood events are predicted to increase in many regions of the world. This is expected to cause large changes in soil functioning and to a progressive decline in soil quality such as reduced rates of nutrient cycling, enhanced greenhouse gas emissions and loss of soil biodiversity. There is a knowledge gap, however, on how temperate agricultural soils under different management practices (e.g. manure application) respond to prolonged river or coastal flooding. The main objective of this work was to determine the effects of a simulated prolonged flooding with saline and freshwater on soil N cycling, following application of a low C:N organic amendment (broiler litter) at two temperatures, representative of a winter and a spring flood event. Using laboratory mesocosms we simulated prolonged winter (6 °C) and spring (14 °C) flooding of soil amended with broiler litter. We also compared the effects of inundation with either river (freshwater) or coastal (saline) water. An agricultural grassland soil (Eutric Cambisol) was subjected to different combinations of treatments (flood with fresh or saline water, winter vs spring temperatures, with/without poultry manure). The impact of these treatments on soil solution N dynamics, greenhouse gas emissions (CO₂, CH₄, N₂O) and microbial community structure (by PLFA analysis) were evaluated over an 11 week simulated flood event followed by an 8 week soil recovery period (without flood). Overall, potential losses of NH₄⁺ and cumulative GHG emissions were increased by flooding and the presence of manure. CH₄ emissions were found to dominate under freshwater flooding conditions and N₂O under saline flooding. Significant releases of GHG occurred during both flooding and after floodwater removal. Temperature was less influential on regulating GHG under the different treatments. These releases in GHG were associated with disruption in N cycling and changes in soil microbial composition and these changes persisted after floodwater removal. Extreme flooding negatively impacts soil functioning, however, the magnitude of any changes remain critically dependent on flood duration and source of flood water, and management conditions. Further work is required at the field scale to understand the molecular basis of the responses observed in this study.

1. Introduction

Changes in global weather patterns and the increased incidence of extreme events in recent years are starting to negatively impact on the sustainability of agricultural ecosystems (IPCC, 2013). Although storms and extreme flood events are not rare, evidence suggests that their frequency and magnitude is increasing in many regions of the world (Pohl et al., 2017; WMO, 2013). These are exemplified by recent extreme flood events in many parts of Europe (Met Office, 2014; Romanescu and Stoleriu, 2017). Typically, these are triggered by prolonged heavy rainfall, however, they are being further compounded in coastal regions by global sea level rise and tidal surges (Haigh et al.,

2016; Nicholls et al., 1999). In some cases, flooding affects soils with a known history of waterlogging (e.g. Fluvisols, Gleysols) and the consequences may not be too severe, however, areas with no previous history of flooding are also becoming affected. For example, in the extreme winter storms of 2014, floodwater covered large areas of agricultural land in the UK for up to 3 months with floodwater depths exceeding 2 m, ultimately leading to the loss of crops and excessive soil erosion (Defra, 2014; Sibley et al., 2015; Smith et al., 2017). These extreme events have the potential to cause irreversible damage to plant growth, agricultural productivity and ecosystem functioning (Niu et al., 2014), as a result of disruptions in soil physical structure, nutrient cycling (Baldwin and Mitchell, 2000; Scalenghe et al., 2012) and soil

Abbreviations: GHG, greenhouse gas; PLFA, phospholipid fatty acid

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microbial function (Bossio and Scow, 1998).

The nitrogen (N) cycle may be particularly affected by extreme flooding due to its capacity to reduce soil O₂ concentrations and the subsequent effects this has on key soil processes such as N mineralisation, immobilisation, nitrification and denitrification as well as plant N uptake (Herzog et al., 2016). Typically, prolonged waterlogging can cause the accumulation of NH₄⁺ in soil (White and Reddy, 2009), stimulate NH₃ volatilization (Zhong-Cheng et al., 2012), whilst any NO₃⁻ present in the soil at the time of flooding may be denitrified and represent a source of N₂O emissions (Granli and Bockman, 1994). Prolonged flooding also has the potential to increase leaching of residual soil nitrate depending on the nature of the flood event (i.e. surface-versus ground-water driven floods; Huber et al., 2012).

The relative contribution of different anthropogenic activities to the increased incidence of flooding remains uncertain, however, it has been directly linked to shifts in land use, increased urban run-off, river network engineering and indirectly to increased greenhouse gas (GHG) emissions (i.e., methane-CH₄, carbon dioxide-CO₂ and nitrous oxide-N₂O) (Pall et al., 2011). As the agricultural release of CH₄ and N₂O are frequently stimulated under waterlogging conditions, there is potential for a positive feedback on climate change and flood risk. In addition, N₂O may be released following floodwater removal as the block in nitrification is removed or during partial denitrification (Norton, 2008; Robertson and Groffman 2007).

There are numerous studies on the effects of flooding on N and carbon (C) cycling in rice paddy fields (Nguyen et al., 2015; Peng et al., 2011; Pereira et al., 2013; Zhang et al., 2012; Zhang et al., 2015), riparian zones (Baldwin and Mitchell, 2000) and wetlands (Unger et al., 2009; Wang et al., 2013). In contrast, however, there are fewer published studies on the impacts of extreme flooding for temperate agricultural soils, particularly those with no previous history of flooding and under contrasting management regimes (Hansen et al., 2014).

We hypothesize that one of the agricultural practices that is most likely to influence how soil quality responds to flooding is the presence of nutrient-rich organic material with different N mineralization rates (e.g. animal manures or green cover crops; Masunga et al., 2016). Our rationale is that these fertilisers are typically applied to soil before crop establishment when winter/spring flooding occurs and they are well known to promote shifts in microbial community functioning and greatly influence net GHG emissions (Snyder et al., 2009). Further, we hypothesized that the outcome will be greatly influenced by floodwater type. We predict that saline coastal flooding will emit less GHG due to the high concentration of alternative electron acceptors in seawater (28 mM SO₄²⁻) relative to freshwater (ca. < 0.1 mM SO₄²⁻), and also to the negative impact of excess NaCl on microbial activity. Our aim was therefore to determine the effects of simulated prolonged flooding with saline or freshwater on soil N cycling, following the application of a low C:N organic amendment (broiler litter), which could accelerate or slow the decomposition of soil organic matter (Liu et al., 2017) at two temperatures, representative of a winter and a spring flood. Our main objectives were to investigate alterations in (1) N cycling, including changes in soil water chemistry and GHG emissions (CH₄, CO₂ and N₂O), and (2) soil microbial structure at the end of flooding (11 weeks), and after the soil recovery period (8 weeks).

2. Materials and methods

2.1. Soil, water and manure properties

Replicate soil samples (5–20 cm depth; Ah horizon, Eutric Cambisol) were collected from a low intensity sheep (*Ovis aries* L.) grazed grassland dominated by *Lolium perenne* L. located at the Henfaes Experimental Station, Abergwyngregyn, UK (53°14'19"N, 4°00'55"W; altitude 18 m a.s.l.). The mean annual temperature at the site is 10 °C and the mean annual rainfall is 960 mm. Within living memory, the sampling site has not previously been flooded, however, the

surrounding region has recently been subjected to both unprecedented river and coastal flooding (Sibley et al., 2015; see Supplementary Information, Fig. A.1 and Fig. A.2). Prior to use, the soil was coarse-sieved (1 cm mesh) to remove any discernible roots and stones, maintain the soil's crumb structure and minimize changes in microbial activity and N cycling (Jones and Willett, 2006). Particle size was analyzed according to Gee and Bauder (1986) while total C and N was determined using a Truspec[®] CN Elemental Analyser (Leco Corp, St Joseph, MI). Plant-available P and K were determined by extracting the soil with 0.5 M acetic acid (1:5 w/v; 1 h, 200 rev min⁻¹), centrifuging the extracts (10,000 g, 10 min) and analyzing P by the molybdate blue method of Murphy and Riley (1962) and K by flame photometry using a Sherwood Scientific Flame Photometer (Fisher Scientific, Loughborough, UK). Soil pH and electrical conductivity (EC) were determined in (1:2.5 v/v) soil:distilled water extracts using standard electrodes.

Two different sources of floodwater were used in the experiment: (1) freshwater from the nearest large watercourse (Rhaeadr-fawr river, 53°14'8"N, 4°0'59"W; located ca. 350 m away from the soil sampling site), and (2) seawater, from the adjacent Menai Strait (53°14' 20"N, 4°1'54"W; located ca. 700 m away from the soil sampling site). Floodwater pH and EC were measured directly using standard electrodes, while NH₄⁺ was determined colorimetrically using the salicylate method of Mulvaney (1996) and NO₃⁻ using the vanadate method of Miranda et al. (2001) using an Epoch[®] microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT).

Broiler litter was collected from a commercial poultry farm on Anglesey, North Wales (53°15'N, 4°18'W). Its dry matter content was determined by oven drying (105 °C, 48 h). Its total C and N content, NH₄⁺ and NO₃⁻ were determined on fresh material as detailed above. Total P and total K were analysed in dry manure (105 °C, 24 h) after sieving to pass a 1 mm screen and digesting with aqua-regia (EPA, 1996). Manure pH was determined in a 1:6 (w/v) manure:distilled water extract using standard electrodes while uric acid was determined according to Cox et al. (1996). After collection, all soil, floodwater and manure were kept refrigerated at 4 °C until required.

2.2. Experimental treatments and stages of the experiment

Transparent polypropylene containers (11 × 8 cm base, 27 cm high; *n* = 48) were filled with 850 g of sieved field-moist soil to achieve a bulk density of 1 g cm⁻³ based on field measurements of the soil in situ. Broiler litter (8 g mesocosm⁻¹, equivalent to 9.1 t ha⁻¹ on a surface area basis) was then mixed by hand with the soil in half of the mesocosms. The rate was chosen to reflect those typically used on UK grasslands (Defra, 2010). Broiler litter was chosen based on its widespread use for improving soil quality and its presence in fields impacted by the UK's 2014 extreme floods. Overall, the experiment had 4 main treatments:

- Soil only (Control)
- Soil + flooding
- Soil + manure
- Soil + manure + flooding

The secondary factors in the experiment were floodwater type (freshwater vs. saline) and temperature regime (6 °C vs. 14 °C) giving 48 mesocosms in total. A Rhizon[®] soil water sampler (Rhizosphere Research Products, Wageningen, The Netherlands) was placed in the centre of the soil at an angle of 45° in each mesocosm prior to the addition of floodwater. Two hundred and fifty ml of fresh or saline floodwater was carefully added to the soil surface in all mesocosms to achieve field capacity – these were defined as the non-flooded treatments. For the 'flooded' treatments, additional floodwater (ca. 1 l) was added to half the mesocosms to achieve a flood depth 9 cm above the soil surface. This reflected field observations of typical flood depths within the region. Finally, the boxes were randomized and placed in climate-control rooms in the dark at either 6 °C (simulated winter) or 14 °C (simulated spring) and loosely covered with polythene to

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