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Short communication

Freeze-thaw and dry-wet events reduce microbial extracellular enzyme activity, but not organic matter turnover in an agricultural grassland soil

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ABSTRACT

Soils in temperate agroecosystems are frequently exposed to extremes of moisture and temperature during which time soil functioning may be negatively affected. The aim of this study was to directly compare the effects of a single dry-wet or freeze-thaw (−5 °C or −20 °C) cycle on extracellular enzyme activity and soil organic matter turnover. We measured the activity of six enzymes before and after imposing the freeze-thaw or dry-rewet events. Our results showed that drying had a much greater impact on total enzyme activity than a −20 °C freezing event (38 vs. 10% reduction, respectively), while freezing at −5 °C had no appreciable effect. Enzyme activity recovered back to control levels relatively quickly which we ascribe to de novo exoenzyme production (within 3 d for the −20 °C freeze-thaw treatment and 14 d for the dry-wet treatment). We added ^{14}C-labelled plant residues to the soil prior to imposing the same thermal or moisture stress events. Monitoring residue decomposition before and after imposing the treatments indicated that none of the stress regimes greatly affected organic matter turnover rates. Our results did reveal, however, a pulse of $^{14}CO_2$ which was produced during the drying and freezing events themselves. We ascribe this to a shift in microbial metabolism and the production of stress avoidance metabolites (e.g. osmo- and cryo-protectants, membrane lipids). Our work highlights that extreme weather events may affect exoenzyme activity, however, these responses are transitory and are unlikely to greatly affect soil organic matter cycling unless they occur at high frequency.

Microbial degradation of organic matter plays an important role in biogeochemical cycling within agroecosystems. The primary stages of soil organic matter (SOM) turnover involves the production of extracellular enzymes by soil microbes and, to a lesser extent plant roots (Burns et al., 2013). These exoenzymes catalyze the breakdown of high molecular weight (MW) plant and microbial derived-polymers into soluble products which can then be rapidly assimilated by soil microbes and/or plants. They also facilitate the release of inorganic nutrients (e.g. N, P; Allison et al., 2006). As soil exoenzyme activities often represent the rate-limiting step in SOM turnover, they can provide a good indicator of biochemical processes operating within the soil ecosystem, SOM characteristics, and nutrient limitation (Allison et al., 2006).

Freeze-thaw or dry-wet events are known to frequently occur in many agricultural soils. When temperatures fall below 0 °C, ice crystals can entrap and/or denature extracellular enzymes retarding their activity. Similarly, drying of soil causes enzymes to become confined in thin water films, inducing denaturation or sorption to the solid phase. A number of studies have reported that freeze-thaw or dry-wet events can affect enzyme activities, however, these reports are often contradictory showing either increases (Tabatabai and Bremmer, 1970; Sistla and Schimel, 2013), decreases (Lee et al., 2007; Turner and Romeo, 2010), or no net change in activity (Bandick and Dick, 1999).

Freeze-thaw or dry-wet events have often been shown to induce a significant increase in soil CO_2 production. This response has been ascribed to a range of factors including: (i) physical disruption of soil aggregates allowing access to previously trapped carbon (C) (Denef et al., 2001); (ii) death and lysis of microbial cells and mesofauna followed by the subsequent breakdown of this necromass when the moisture or temperature regime returns to normal; (iii) increased microbial metabolism and the use of energy during the production of stress compounds (e.g. cryo- and osmo-protectants), and (iv) changes in pH and ionic strength which increases SOM solubilization (Edwards and Cresser, 1992; Fierer and Schimel, 2002; Schimel et al., 2007).

The aim of this study was to directly compare the exoenzyme response of an agricultural soil to either a dry-wet or freeze-thaw event. In addition, we aimed to test whether any changes in the exoenzymes profile affected the turnover of plant residues present in soil. As both freeze-thaw and dry-wet events may affect the microbial biomass in

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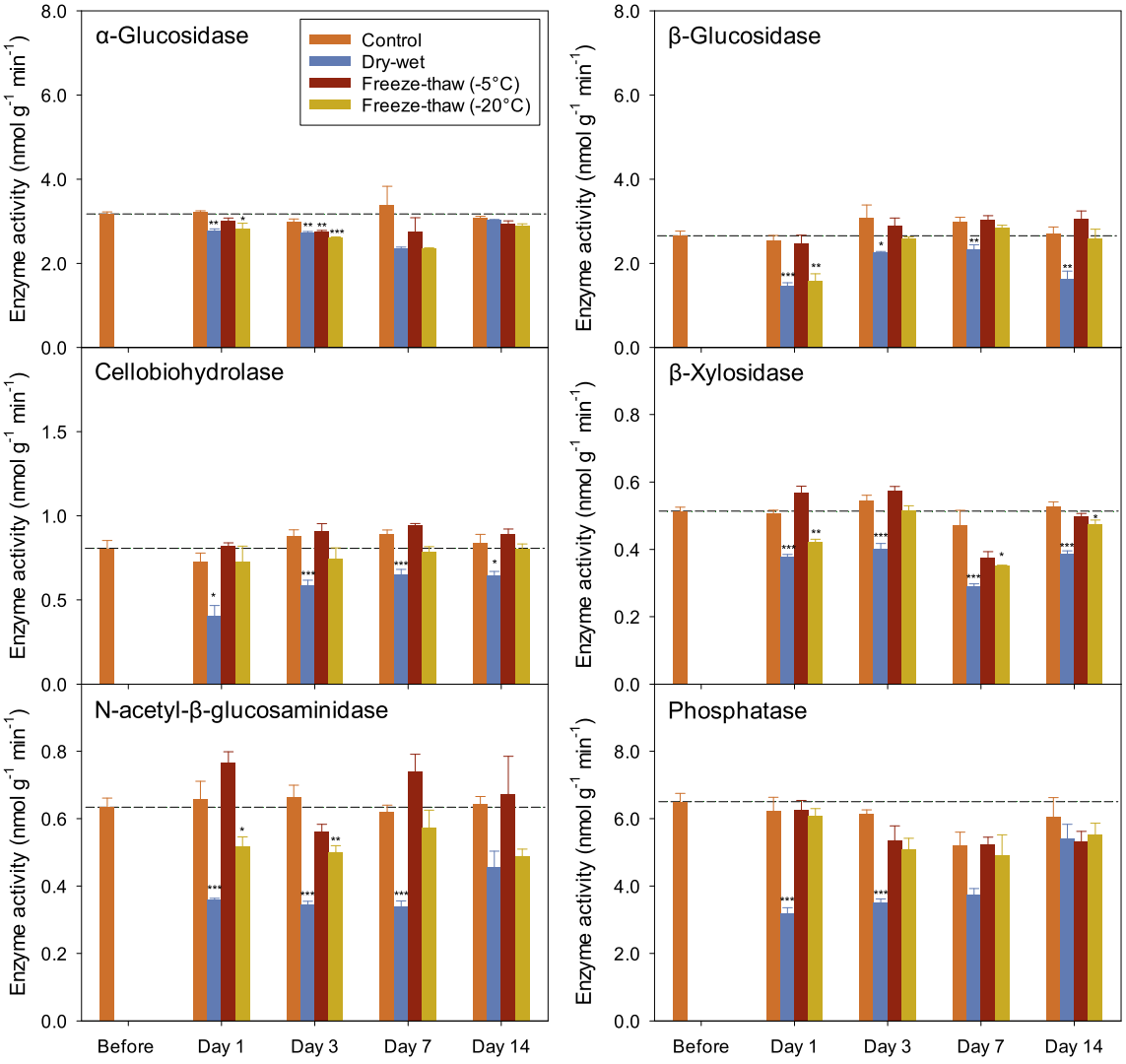

Fig. 1. Activity of six extracellular enzymes in soil before and after the application of a freeze-thaw (−5 °C or − 20 °C) or dry-wet event. The legend is the same for all panels. The dotted line represents the enzyme activity before applying the treatment. Stars above the plots denote significant differences from the control where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively. Values represent means ± SEM ($n = 4$).

similar ways (inducing the production of stress compounds or causing cell lysis), we hypothesized that they would both reduce exoenzyme production. We predict that this will affect de novo enzyme production. As most exoenzymes are quite persistent in soil, we further hypothesized that the intrinsic enzymes would be denatured to a greater extent under drying than freezing (due to the enzymes becoming sorbed to the solid phase or precipitating with humic substances concentrated in the thin water films). We predict that this will negatively affect the turnover of native soil organic matter.

Soil samples (5–10 cm depth, Ah horizon) were collected from a sandy clay loam textured Eutric Cambisol in a sheep-grazed, *Lolium perenne* L. dominated grassland located at the Henfaes Experimental Station, Abergwyngregyn, UK (53°14′22″N, 4°00′60″W). The mean annual air temperature is 10.6 °C (max 28.6 °C, min −7.6 °C) and the mean annual rainfall is 1055 mm y^{-1}. The lowest temperature ever recorded in the region was −23 °C in 1940. The mean winter air temperature is 5 °C (Fig. S1). The grassland receives regular fertilizer at an annual rate of 50 kg N ha^{-1}, 10 kg P ha^{-1} and 10 kg K ha^{-1}.

To characterize the soil, four independent replicates of soil, located 10 m apart, were collected from the field and sieved to pass 2 mm, removing stones, roots and earthworms. The pH (5.47) and electrical conductivity (121 μS cm^{-1}) were determined in a 1:5 (*w/v*) soil-distilled water extracts. Soil moisture (36.4%) and organic matter content (6.53%) were determined gravimetrically by oven-drying the soil for 24 h at 105 °C and 450 °C, respectively. Dissolved organic carbon (30.9 mg L^{-1}) in soil solution (1:1 w/v soil-distilled water extract) was determined using a multi N/C 2100 analyzer (Analytik-Jena AG, Jena, Germany).

The experiments were set up to reflect winter conditions and therefore the soil was maintained at 5 °C until the start of the experimental treatments. Either a single freeze-thaw (−5 °C or −20 °C) or dry-wet event was applied to the soil. For the freeze-thaw treatments, the samples were placed in an incubator at either −5 °C or −20 °C for 24 h. After freezing, the samples were allowed to thaw by placing them back at +5 °C. For the dry-wet treatment, air was passed over the samples at a rate of 0.86 m s^{-1} until they had reached an air-dry state (ca. 3 h; Fig. S2). After drying (24 h), distilled water was added back to the soil to reach the pre-drying water content (5 °C). Control samples were maintained at 5 °C throughout the experiment. Each treatment had 4 independent replicates.

The activity of six hydrolytic soil extracellular enzymes (α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylosidase, *N*-acetyl-β-glucosaminidase, acid phosphatase; Table S1) were measured following the 96-wellplate fluorometric method described in Freeman et al. (1995)

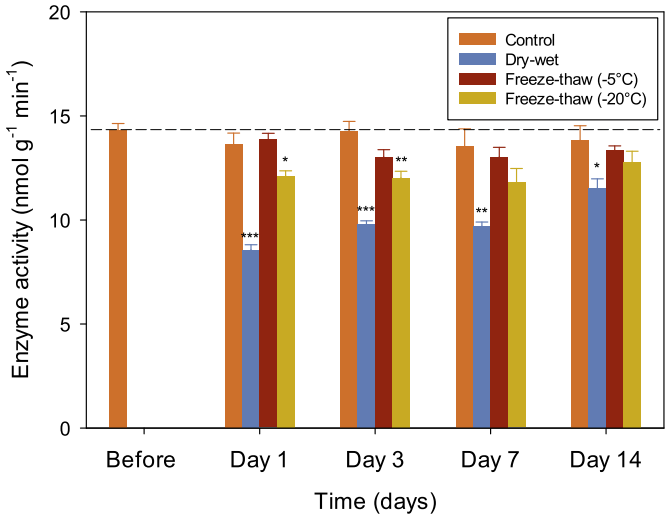

Fig. 2. Total hydrolytic extracellular enzyme activity in soil before and after the application of a freeze-thaw (−5 °C or − 20 °C) or dry-wet event. The dotted line represents the enzyme activity before applying the treatment. Stars above the plots denote significant differences where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively. Values represent means ± SEM ($n = 4$).

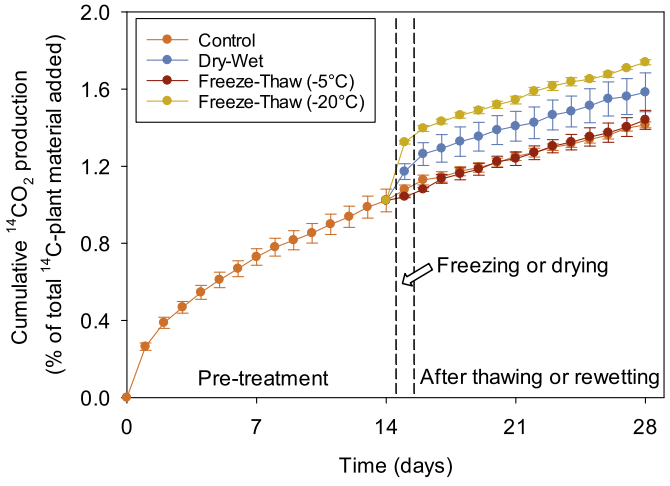

Fig. 3. Cumulative $^{14}CO_2$ production following addition of ^{14}C-labelled plant material to soil before and after the application of a freeze-thaw (−5 °C or − 20 °C) or dry-wet event. Values represent means ± SEM ($n = 16$ for pre-treatments and $n = 4$ after thawing or rewetting). The dotted lines denote the times at which the conditions were changed.

and Dunn et al. (2014). Enzyme activity was measured both immediately before imposing the freezing or drying treatments and then 1, 3, 7 and 14 d after thawing or rewetting, respectively. Briefly, the fluorogenic 4-methylumbelliferyl (MUF)-labelled substrates were dissolved in methyl cellosolve® (ethylene glycol monomethyl ether) before being diluted in ultrapure water. No pH buffer was used in the assays. Soil (1 g) was placed in a stomacher® bag (Seward Ltd., West Sussex, UK), 7 ml of the relevant MUF substrate added and the sample mixed in a laboratory paddle blender (Stomacher® circulator, Seward Ltd.) for 30 s. The samples were then incubated (5 °C, 60 min), after which they were centrifuged (18,000 *g*, 5 min) and the supernatants placed into 96 well plates and their fluorescence quantified using a SpectraMax M2e fluorimeter (Ex. 350 nm, Em. 465 nm; Molecular Devices Inc., San Jose, CA).

To generate the ^{14}C-labelled plant residues we pulse-labelled *Lolium perenne* L. (12.3 kBq g^{-1}) with $^{14}CO_2$ as described in Hill et al. (2007). Subsequently, we isolated the ^{14}C-labelled high MW insoluble fraction from the shoots (e.g., cellulose, hemicellulose, protein, lignin) as described in Glanville et al. (2012). Briefly, the soluble ^{14}C component (33 ± 2% of the total ^{14}C) was removed from the plant material by extracting the shoots twice with hot water (80 °C). The remaining plant residues were oven-dried (80 °C, 24 h) prior to use in the experiments.

To determine the mineralization of the high MW plant residues, field-moist soil (1 g) was placed in a 50 cm^3 polypropylene tube. Subsequently, 20 mg of the ^{14}C-labelled plant residues were mixed with the soil. A vial containing NaOH (5 M, 200 µl) was then suspended above the soil to capture any evolved $^{14}CO_2$. After sealing, the tubes were placed in an incubator in the dark at 5 °C and the NaOH traps replaced daily. After 14 d, the soils were exposed to a freezing or drying event for 24 h as described above. After thawing or rewetting, the samples were further incubated at 5 °C for 14 d during which time the NaOH traps were replaced daily. After dilution (1:5 v/v), the amount of ^{14}C in the NaOH traps was determined by liquid scintillation counting using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keyes, UK) and HiSafe 3 scintillation fluid (PerkinElmer Inc., Waltham, MA).

A one-way analysis of variance (ANOVA) was performed to determine the effect of freeze-thaw or dry-wet treatments on enzyme activity and $^{14}CO_2$ evolution in soils at each time point. To compare differences within groups, multiple comparisons were carried out by Tukey post-hoc testing at a significance level of $p < 0.05$. Repeated measures ANOVA was undertaken to evaluate differences in cumulative $^{14}CO_2$ evolution between treatments. Statistical analysis was undertaken in R Studio 0.99.486 (R Development Core Team, 2004).

Overall, a dry-wet cycle had a greater effect on soil exoenzyme activity than a freeze-thaw cycle ($p < 0.001$; Fig. 1). In the case of drying, the activity of all six exoenzymes were reduced, with the biggest response seen directly after the soil was rewetted (i.e. within 24 h). In some cases, the negative impact of drying persisted for up to 14 d after rewetting (e.g. β-xylosidase, β-glucosidase). When all six enzymes were considered together, a dry-wet cycle caused an initial reduction in enzyme activity of 38%, however, these activities progressively recovered over time but were still lower at day 14 in comparison to the control (ca. 20%; Fig. 2).

In the case of a freeze-thaw event, the reduction in enzyme activity was generally more severe at −20 °C in comparison to soils exposed to −5 °C (Fig. 1). In contrast to the drying treatment, some enzymes were unaffected by freezing (e.g. acid phosphatase, cellobiohydrolase), while others were affected but to a lesser extent (e.g. α- and β-glucosidase). In most cases, levels of enzyme activity recovered quickly after freezing with few differences detected after 3 d of thawing in comparison to the unfrozen control. When total enzyme activity was considered, no significant differences to the control were observed for the −5 °C treatment, while only a 10% reduction was observed for the −20 °C treatment (Fig. 3).

Overall, the rate of plant residue mineralization was slow with only 1.4% of the plant material being mineralized in the control treatment over the 30 d incubation period (Fig. 3). The rate of mineralization was not affected by freezing the soil at −5 °C. In contrast, the amount of $^{14}CO_2$ recovered in both the dry-wet and − 20 °C freezing treatments were higher than observed in the control ($p < 0.001$). Most of this additional $^{14}CO_2$, however, was produced during the stress event itself, with no major difference seen after this point (Fig. 3).

Relative to the conditions at the study site, our results show that exposure to mild freezing (−5 °C) had no appreciable effect on enzyme activity, while exposure to extreme freezing (−20 °C) caused a transient reduction in enzyme activity. These results are consistent with other studies assaying different exoenzymes in contrasting ecosystem and soil types (Li et al., 2012; Mannisto et al., 2018; Sorensen et al., 2018). The initial reduction in enzyme activity at −20 °C are probably related to protein aggregation, binding to humic substances, sorption to the solid-phase, and ice-induced conformational changes, all of which are known to affect catalytic rate (Champion et al., 2000; Cao et al.,

2003; Terefe et al., 2004; Nardid et al., 2014). The lack of effect at −5 °C could be due to the protection of exoenzymes and microbial cells in unfrozen liquid water (Edwards and Cresser, 1992; Brooks et al., 1997). Our results also show that upon thawing, enzyme activity recovered quickly to those seen in the unfrozen control soil. Interestingly, at no time did their levels exceed those in the controls, suggesting that the freezing event did not greatly stimulate microbial activity or make C substrates more bioavailable. This is also consistent with the lack of effect seen on the mineralization of the ^{14}C-labelled plant residues which showed no change in mineralization rate after thawing. The apparent increase in $^{14}CO_2$ production during the freezing event itself we ascribe to plant-derived ^{14}C that was now immobilized in the microbial biomass and which is rapidly respired due to changes in metabolic activity in an effort to protect against extreme temperatures (e.g. changes in membrane lipid composition and production of cryoprotectants; Feng et al., 2007; Marx et al., 2008).

In contrast to freeze-thaw, a dry-wet cycle had a much greater impact on enzyme activity. In our experience, these drying events are much more frequent than freezing at our field site, especially at the soil surface (0–1 cm). Our finding that drying reduces enzyme activity and that recovery occurs within 2 weeks are consistent with studies in other ecosystems (Pohlon et al., 2013; Frossard et al., 2015). They do, however, contrast with others which have shown an increase in enzyme activity following rewetting, a response that has been ascribed to endoenzyme release during cell lysis (Tabatabai and Bremmer, 1970; Zhao et al., 2010; Burns et al., 2013). The slow recovery of enzyme activity in comparison to freeze thaw may suggest that the microbial community was affected to a greater extent (i.e. greater cell lysis). However, the observation that the mineralization of the ^{14}C-plant residues was not affected by drying strongly suggests that this may not be the case. Rather, the peak in $^{14}CO_2$ during the drying event we ascribe to changes in microbial metabolism upon imposition of the moisture stress (e.g. osmoprotectant production). This is also consistent with Magid et al. (1999) who showed no effect on SOM turnover. We therefore hypothesize that although exoenzyme rates were reduced by drying that this is not the rate limiting step in SOM breakdown, at least for the enzymes measured here. As many hundreds of enzymes are involved in SOM turnover, further work is needed to ascertain if our findings are consistent across other functional enzyme groups (e.g. those involved in protein, lipid and lignin degradation). The recovery of exoenzyme activity back to control levels within 14 d also indicates that the de novo production of exoenzymes by the microbial community is relatively rapid (Burns, 1982).

In conclusion, this study shows that extracellular enzymes were relatively insensitive to a single freeze-thaw event but were greatly affected by a single dry-wet event. In both cases, enzyme activity recovered quickly after removal of the stress. The fact that enzyme activities never increased above the control levels, and that the decomposition of plant residues was unaffected by dry-wet and freeze-thaw, suggest that these extreme weather events have minimal effect on SOM turnover. Our evidence, however, does suggest that the microbial community rapidly responds to the stress event itself (drying/freezing) by increasing metabolism, however, further work examining the metabolic responses of the microbial community are required to confirm this.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.08.002.

References

Allison, S.D., Gartner, T., Holland, K., Weintraub, M., Sinsabaugh, R., 2006. Soil enzymes: linking proteomics and ecological process. Environ. Microbiol. 3, 704–711.
Bandick, A., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479.
Brooks, P.D., Schmidt, S.K., Williams, M.W., 1997. Winter production of CO_2 and NO_2 from alpine tundra: environmental controls and relationship to inter-system C and N fluxes. Oecologia 110, 403–413.
Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem. 14, 423–427.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.
Cao, E., Chen, Y., Cui, Z., Foster, P.R., 2003. Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. Biotechnol. Bioeng. 82, 684–690.
Champion, D., Blond, G., Le Meste, M., Simatos, D., 2000. Reaction rate modeling in cryoconcentrated solutions: alkaline phosphatase catalysed DNPP hydrolysis. J. Agric. Food Chem. 48, 4942–4947.
Denef, K., Six, J., Paustian, K., Merckx, R., 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry-wet cycles. Soil Biol. Biochem. 33, 2145–2153.
Dunn, C., Jones, T.G., Girard, A., Freeman, C., 2014. Methodologies for extracellular enzyme assays from wetland soils. Wetlands 34, 7–9.
Edwards, A.C., Cresser, M.S., 1992. Freezing and its effect on chemical and biological properties of soil. Adv. Soil Sci. 18, 59–79.
Feng, X.J., Nielsen, L.L., Simpson, M.J., 2007. Responses of soil organic matter and microorganisms to freeze-thaw cycles. Soil Biol. Biochem. 39, 2027–2037.
Fierer, N., Schimel, J., 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol. Biochem. 34, 777–787.
Freeman, C., Liska, G., Ostle, N.J., Jones, S.E., Lock, M.A., 1995. The use of fluorogenic substrates for measuring enzyme-activity in peatlands. Plant Soil 174, 147–152.
Frossard, A., Ramond, J.B., Seely, M., Cowan, D.A., 2015. Water regime history drives responses of soil Namib Desert microbial communities to wetting events. Sci. Rep. 5, 12263.
Glanville, H.C., Hill, P.W., Maccarone, L.D., Golyshin, P.N., Murphy, D.V., Jones, D.L., 2012. Temperature and water controls on vegetation emergence, microbial dynamics, and soil carbon and nitrogen fluxes in a high Arctic tundra ecosystem. Funct. Ecol. 26, 1366–1380.
Hill, P.W., Marshall, C., Williams, G.G., Blum, H., Harmens, H., Jones, D.L., Farrar, J.F., 2007. The fate of photosynthetically-fixed carbon in *Lolium perenne* L. grassland as modified by elevated CO_2 and sward management. New Phytol. 173, 766–777.
Lee, Y.B., Lorenz, N., Dick, L.K., Dick, R.P., 2007. Cold storage and pretreatment incubation effects on soil microbial properties. Soil Sci. Soc. Am. J. 71, 1299–1305.
Li, Z.H., Xu, H.F., Tang, J., Li, Z.Y., Zhang, N., Liang, S., 2012. Response of microbial biomass carbon, community and activity to freeze-thaw cycles of saline-alkali soil. Fresenius Environ. Bull. 21, 3847–3852.
Magid, J., Kjaergaar, C., Gorissen, A., Kuikman, P.J., 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added ^{14}C-labelled plant material. Soil Biol. Biochem. 31, 595–602.
Mannisto, M., Vuosku, J., Stark, S., Saravesi, K., Suokas, M., Markkola, A., Martz, F., Rautio, P., 2018. Bacterial and fungal communities in boreal forest soil are insensitive to changes in snow cover conditions. FEMS Microbiol. Ecol. 94 (fiy123).
Marx, J.G., Carpenter, S.D., Deming, J.W., 2008. Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychrerythraea* strain 34H under extreme conditions. Can. J. Microbiol. 55, 63–72.
Nardid, O.A., Rozanova, E.D., Naumenko, E.I., 2014. Freeze-thawing induced structural and functional changes in glucose oxidase. Cryoletters 35, 114–118.
Pohlon, E., Fandino, A.O., Marxsen, J., 2013. Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting. PLoS One 8, e83365.
R Development Core Team, 2004. *R: a language and environment for statistical computing.* Version 2.0.1. R Foundation for Statistical Computing, Vienna, Austria.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecol 88, 1386–1394.
Sistla, S.A., Schimel, J.P., 2013. Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: identifying direct and indirect effects of long-term summer warming. Soil Biol. Biochem. 66, 119–129.
Sorensen, P.O., Finzi, A.C., Giasson, M.A., Reinmann, A.B., Sanders-DeMott, R., Templer, P.H., 2018. Winter soil freeze-thaw cycles lead to reductions in soil microbial biomass and activity not compensated for by soil warming. Soil Biol. Biochem. 116, 39–47.
Tabatabai, M.A., Bremmer, J.M., 1970. Factors affecting soil arylsulfatase activity. Soil Sci. Soc. Am. J. 34, 427–429.
Terefe, N.S., Arimi, J.M., Van Loey, A., Hendrickx, M., 2004. Kinetics of the alkaline phosphatase catalyzed hydrolysis of disodium p-nitrophenyl phosphate: effects of carbohydrate additives, low temperature, and freezing. Biotechnol. Prog. 20, 1467–1478.
Turner, B.L., Romeo, T.E., 2010. Stability of hydrolytic enzyme activity and microbial phosphorous during storage of tropical rain forest soils. Soil Biol. Biochem. 42, 459–465.
Zhao, B.Z., Chen, J., Zhang, J.B., Qin, S.W., 2010. Soil microbial biomass and activity response to repeated drying-rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. Geoderma 160, 218–224.