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Miura, Maki; Jones, Timothy G.; Ford, Hilary; Hill, Paul W.; Jones, Davey L.

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Role of plants in determining the soil response to either a single freeze-thaw or dry-wet event

Maki Miura[a,b,*], Timothy G. Jones[a], Hilary Ford[a], Paul W. Hill[a], Davey L. Jones[a,c]

[a]*School of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK*

[b]*Graduate School of Global Environmental Studies, Kyoto University, Kyoto, 606-8502, Japan*

[c]*SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University, Murdoch WA 6105, Australia*

Email: Maki Miura bsp620@bangor.ac.uk

ORCID: 0000-0001-6976-4541

Abstract

In a changing climate, extreme weather events are predicted to increase in frequency and magnitude. These events may induce stress in plants and soil microbial communities, but the impact of climate extremes on root-soil interactions remains poorly understood. To better understand the response of a temperate agroecosystem to winter freezing and drought, a mild (-5°C) or severe (-10°C) freeze-thaw, or dry-wet cycle was imposed on mesocosms planted with winter wheat (*Triticum aestivum* L.) and unplanted soil. We measured the effect of the stresses on greenhouse gas (GHG) fluxes (CO_2, N_2O, CH_4), plant tissue composition, soil solute concentrations and soil microbial community structure. Only the most severe freezing event had a direct effect on soils, with pulses of CO_2 and N_2O released after thawing. In contrast, all stresses reduced C fixation and respiration in planted treatments. Total CO_2 flux from planted mesocosms was reduced during the drought period and CO_2 flux was negatively correlated with soil water content. The severe freeze-thaw event caused lasting damage to plants and increased rhizodeposition, resulting in increased CO_2 efflux and a small alteration in soil microbial community composition. The presence of plants resulted in significantly greater total CO_2 flux following freeze-thaw or dry-wet events, but only in unplanted soil was there a net increase in GHG emissions. These results demonstrate that, although the effects of stress appear magnified where plants are present, the maintenance of winter plant cover in temperate agricultural soils reduces the effects of extreme weather events on future climate.

Keywords: Climate change, carbon cycle, sustainable agriculture, nitrogen cycle, nutrient dynamics.

Declarations of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Climate change is likely to alter the frequency of freeze-thaw or dry-wet events with wide-reaching implications for the functioning of temperate agroecosystems (Kreyling et al., 2007; Donat et al., 2016). Freeze-thaw or dry-wet events are important ecological processes that affect soil hydrology and thermodynamics (Harrison-Kirk et al., 2013; Yang and Wang 2019), and can trigger stress responses in both plants (Harp et al., 2010; Skinner, 2015) and soil microbes (Schimel et al., 2007). Freezing and drying processes can damage soil microbial and root cells, releasing organic carbon (C) and nitrogen (N), which results in an increase in soil solution concentrations (Schimel et al., 2007). These changes in soil C and N concentration may directly affect native soil organic matter (SOM) turnover rates, which may stimulate greenhouse gas (GHG) emissions (Kim et al., 2012). Field and laboratory studies of the effects of freeze-thaw or dry-wet events on soil gas fluxes have been widely reported (Kim et al., 2012), with an increase in CO_2, N_2O, and CH_4 following thawing or rewetting, commonly observed (Xiang et al., 2008; Wu et al., 2010 Kim et al., 2012). The predicted increase in the frequency and magnitude of freeze-thaw or dry-wet events may therefore have important implications for the net GHG budget of agroecosystems.

The root system plays an important role in the response of the soil environment to freeze-thaw or dry-wet events (Whitmore and Whalley 2009; Hosokawa et al., 2017). Root exudates are primarily derived from recently assimilated C and represent a semi-continuous input of labile C to soil (Jones et al., 2009). Most exudates consist of sugars, amino acids, and organic acids (Badri and Vivanco, 2009; Carvalhais et al., 2011) and these low molecular weight (MW) organic compounds can be released into soil by passive diffusion under non-stressed conditions (Jones et al., 1998). The release of organic

67 compounds from roots is a key factor in mediating nutrient availability in the rhizosphere
68 (Pierret et al., 2007) and stimulating microbial activity (Meier et al., 2017). Freeze-thaw
69 or dry-wet events may induce root activity or damage and enhance rhizodeposition, which
70 in turn may affect microbial activity and subsequently soil GHG emissions (Bais et al.,
71 2006; Hinsinger et al., 2009).

72 CO_2 release from soil is due to a combination of heterotrophic (microbial) and
73 autotrophic (plant) respiration (Wang et al., 2014). Although separating heterotrophic and
74 autotrophic respiration may help to understand C cycling under extreme environmental
75 change (Casals et al., 2011; Zhou et al., 2009), root-soil interactions under these
76 conditions are rarely characterized. In mesocosms, drought has been shown to increase
77 root respiration in wheat, although the responses are cultivar specific (Liu et al., 2004).
78 However, comparatively little is known about cereal root gas exchange in response to low
79 freezing temperatures. In the field, catching natural freeze-thaw events in temperate
80 grassland is difficult. Although freeze-thaw cycles using buried heating wire have been
81 studied in grassland (Kreyling et al., 2010), methods of simulating freeze-thaw events are
82 not well established. Therefore, to better understand how freeze-thaw or dry-wet event
83 affect soil with plant roots, we conducted a mesocosm experiment under controlled
84 laboratory conditions.

85 The aim of this experiment was (1) to investigate how the C budget of the
86 combined plant-soil system responded to controlled freeze-thaw and dry-wet events, and
87 (2) to discover how planted and non-planted treatments differed in their CO_2 flux response
88 (daytime, night-time, and total). We hypothesized that CO_2 efflux would reduce during
89 simulated drought conditions due to water limitation, but that a large CO_2 pulse would be
90 released into the atmosphere following freeze-thaw events. We also hypothesized that the

effects of freeze-thaw or dry-wet events on GHG fluxes from planted soil would be greater than from unplanted soil due to the release of root-derived C. The conditions used were chosen to reflect soil conditions in autumn versus spring sown cereal crops.

2. Materials and methods

2.1. Sample collection

Soil samples (5-10 cm depth, Ah horizon) under *Lolium perenne* L. were collected from a crumb-structured Eutric Cambisol in a sheep-grazed grassland located at the Henfaes Experimental Station at Abergwyngregyn, Gwynedd, North Wales (53°14'N, 04°01'W). The soil characteristics for this site is shown in Table S1. The mean annual air temperature at the site 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 at the site was −23 °C in 1940. Fertilizer is regularly added to the grassland (50 kg N ha^{-1}, 10 kg P ha^{-1}, 10 kg K ha^{-1}). Prior to use, the soil was sieved to pass 1 cm and any roots and stones removed. Previous experiments in this soil have shown that this large sieve size minimizes changes to soil structure and microbial functioning (Jones and Willett, 2006). pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-distilled water extract (Smith and Doran 1996). Soil moisture and organic matter content were determined by mass loss following heating for 24 h at 105 °C and 450 °C, respectively.

2.2. Experimental design

Four replicates of field moist soil (700 g) were placed into polypropylene containers (11 × 8 cm base, 27 cm high; Lock & Lock Ltd., Seoul, Republic of Korea),

114 totaling 32 containers. All containers were transferred to a climate-controlled plant
115 growth cabinet (CMP6010; Conviron Inc., Winnipeg, Canada) maintained with a 8 h
116 photoperiod, light intensity of 180 μmol m^{-2} s^{-1}, and relative humidity of 75 % during the
117 experiment. Soil moisture was maintained at 20-25 % of field capacity by adding distilled
118 water every 2-3 days during the experiments.

119 To determine the effects of freeze-thaw and dry-wet events on plants, 6 wheat
120 (*Triticum aestivum* L.) seeds (48 h pre-germinated) were planted in each of the 16
121 containers. The remaining 16 containers were left unplanted. Plants were grown in the
122 plant growth cabinet at 15 °C until the plants were 15 cm tall and had 3 fully expanded
123 leaves. At this point the temperature was reduced to 10 °C to simulate winter conditions.

124 The experiment consisted of two main treatments 1) a single freeze-thaw event,
125 or 2) a single dry-wet event. For the freeze-thaw treatments, after a 5-week pre-treatment
126 period, all samples were transferred to incubators at either -5 °C or -10 °C for 24 h to
127 simulate a freezing period. They were then placed back into a growth cabinet at 10 °C
128 and allowed to thaw naturally. The temperature of -5 °C was considered to be realistic for
129 typical winter conditions in the UK. An extreme freezing treatment of -10 °C was used to
130 reflect conditions which occur infrequently, but also because winter wheat plants have a
131 high degree of freezing tolerance, being able to survive from -4 to -15 °C (Gusta et al.,
132 1997).

133 For the dry-wet treatment, after a week-long pre-treatment period (10 °C), the
134 samples were maintained in a winter dry condition by natural-drying (not adding water)
135 in open containers placed in the growth cabinet (10 °C) for 5 weeks. This reflects periods
136 of spring drought recently experienced in the region. Water loss was determined by
137 weighing. The dry-period was assumed to start when the soil water content had reached

50 - 60 % of field capacity. For rewetting of soil, the amount of water lost was calculated (at the end of drought period) and distilled water was added to the soil surface to bring the water content back to the original water content. Overall, the experiment had 8 treatments: 1) Control with plants (+10 °C, constant soil moisture level), 2) Dry-wet with plants (+10 °C), 3) Freeze -thaw (-5 °C / +10 °C) with plants, 4) Freeze-thaw (-10 °C / +10 °C) with plants, 5) Control without plants (+10 °C, constant soil moisture level), 6) Dry-wet without plants (+10 °C), 7) Freeze-thaw (-5 °C / +10 °C)) without plants, 8) Freeze-thaw (-10 °C / +10 °C) without plants.

2.3. Soil greenhouse gas emissions

Greenhouse gas (GHG) samples were taken from the experimental containers (including entire plants, roots and soil). At each sampling time, gas-tight lids containing a Suba-seal® gas sampling port (Sigma-Aldrich Ltd, Poole, UK) were placed on the polypropylene containers. 20 ml gas samples were then taken from the containers' headspace through the Suba-seal septum by syringe at 0 min (T0) and at 60 min (T60). The gas sample was transferred from the syringe to a 20 ml evacuated glass vial. Preliminary checks where samples were taken every 15 mins showed that the production of CO_2 and N_2O from the mesocosms was linear over time. On each occasion, gas sampling took place twice in the same day at daytime (light condition) and night (dark condition). CO_2, N_2O, and CH_4 concentrations in the vials were determined by gas chromatography using a Clarus 500 GC with a Turbomatrix (HS-40) autoanalyzer (PerkinElmer Inc., Waltham, MA). CO_2 and CH_4 were detected by a flame ionization detector (FID) and N_2O by a ^{63}Ni electron capture detector (ECD). The gas flux was calculated according to Dunn et al. (2016), within the linear portion of a standard curve.

Daily cumulative flux was calculated assuming constant flux between two measurements, multiplying hourly flux by the number of hours (8 during light / 16 during dark for planted) and number of days.

2.4. Soil chemical analysis

Soil nutrient availability was estimated according to Jones and Willett (2006). Briefly, 2 g of soil was removed using a 1.5 cm diameter corer to 2 cm depth from the containers and extracted in 10 ml deionized water on a reciprocating shaker (Edmund Buhler GmbH, SM-30, Germany; 200 rev min^{-1}). After shaking for 10 min, extracts were centrifuged (6850 *g*, 5 min) and then filtered through PES 0.45 μm syringe filters (Triple Red; Avidity Science Ltd, Long Crendon, UK). Soil dissolved organic C (DOC) and total dissolved N (TDN) in soil solution were measured using a Multi N/C 2100/2100 analyzer (AnalytikJena AG, Jena, Germany). Anions and cations were measured by ion chromatography analysis using an 850 Professional IC (Metrohm Ltd., Runcorn, UK).

2.5. Plant analysis

At the end of the experiment on day 138, the plants were removed from the soil, separated into shoots and roots, and oven-dried at 60 °C for 72 h. The shoots and the roots were then ground to a fine powder using a MM200 ball mill (Retsch GmbH, Düsseldorf, Germany). Total C and N of shoots and roots tissues were determined using a Truspec CN analyzer (Leco Corp., St Joseph, MI, USA). Major and trace elements in shoots and roots were determined using a S2 Picofox, Total Reflection X-ray Fluorescence (TXRF) spectrometer (Bruker Inc., Billerica, MA, USA).

186 *2.6. Soil microbial community structure analysis*

187 At the end of the experiment, soil microbial community structure was determined
188 by phospholipid fatty acid (PLFA) analysis according to the method of Buyer and Sasser
189 (2012). Briefly, samples (2 g) from each container were freeze-dried and Bligh–Dyer
190 extractant (4.0 ml) containing internal standard was added. Tubes were sonicated in an
191 ultrasonic cleaning bath (10 min, room temperature) before rotating end-over-end (2 h).
192 After centrifuging (10 min) the liquid phase was transferred to clean 13 mm × 100 mm
193 screw-cap test tubes and 1.0 ml each of chloroform and water were added. The upper
194 phase was removed by aspiration and discarded while the lower phase, containing the
195 extracted lipids, was evaporated (30 °C). Lipid classes were separated by solid phase
196 extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well
197 (Phenomenex, Torrance, CA, USA). Phospholipids were eluted with 0.5 ml of 5:5:1
198 methanol:chloroform:water (Findlay, 2004) into 1.5 ml glass vials (Multi-Tier microplate,
199 E&K Scientific, Santa Clara, CA, USA), and the solution was evaporated (70 °C, 30 min).
200 Transesterification reagent (0.2 ml) was added to each vial, the vials were sealed with a
201 PTFE/silicon cap mat (E&K Scientific, Santa Clara, CA, USA), and incubated (37 °C, 15
202 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were added. The chloroform
203 was evaporated just to dryness at room temperature and samples were dissolved in hexane
204 (75 µl). The samples were analyzed with a 6890 gas chromatograph (Agilent
205 Technologies, Wilmington, DE, USA) equipped with autosampler, split–splitless inlet,
206 and flame ionization detector. Fatty acid methyl esters (FAMEs) were separated on an
207 Agilent Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness.
208 Standard nomenclature was followed for PLFA (Frostegård et al., 1993). Taxonomic
209 groups were ascribed to individual PLFAs using the Sherlock® PLFA Method and Tools

Package (PLFAD1; Microbial ID Inc., Newark, DE). Over one hundred fatty acids were identified in the soil samples, however, we only present results from the twenty-nine whose concentration was higher than 0.5% of the total PLFAs, classified per taxonomic group. The fatty acids ascribed to each taxonomic group were: 14:0 iso, 15:0 iso, 15:0 anteiso, 15:1 iso ω 6c, 16:0 iso, 17:0 iso, 17:0 anteiso and 17:1 iso ω9c were used for Gram+ bacteria; 16:1 ω 5c, 16:1 ω7c, 16:1 ω9c, 17:1 ω8c, 17:0 cyclo ω7c, 18:1 ω5c, 18:1 ω7c, 18:1 ω9c and 19:0 cyclo ω7c were used for Gram− bacteria; 16:0 10 methyl, 17:1 ω7c 10 methyl, 18:0 10 methyl and 18:1 ω7c 10 methyl for actinomycetes; 18:2 ω6c for saprotrophic fungi; and 16:1 ω5c as biomarker for putative arbuscular mycorrhizal fungi (Sánchez-Rodríguez et al., 2019).

2.7. Statistical analysis

Effects of plant (with plant vs without plant) was analyzed using two-way analysis of variance (ANOVA) without interactions. Tukey post-hoc test was used to compare treatment means. Pearson's product-moment correlation was carried out between factors to explore relationship between water contents and GHG flux. One-way ANOVA was performed to determine the effect of freeze-thaw or dry-wet events on plant and root chemicals followed by Tukey post-hoc test. Normalized fatty acid PLFA profiles (Frostegård et al., 1993) were analyzed by principal component analysis (PCA). Significance was evaluated by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Statistical analyses were performed using R v.4.1.0 project (R Development Core Team, 2020) in the 'vegan' package (Oksanen et al., 2020).

3. Results

3.1. CO_2 flux for planted versus non-planted soil

Absolute and cumulative CO_2 fluxes of daytime, night-time and daily totals are shown in Figure 1 (positive fluxes indicate CO_2 efflux i.e. respiration; negative fluxes indicate influx i.e. CO_2 fixation). Due to photosynthetic C fixation, the daytime cumulative CO_2 flux from the planted treatments was generally lower than the flux from unplanted, regardless of specific treatment type ($p < 0.001$; Table S5). In comparison, in both the planted and unplanted treatments the cumulative CO_2 flux during the longer (16 h) night-time period was higher than in the daytime, but the cumulative CO_2 flux from planted treatments was higher than the unplanted soil ($p < 0.001$; Table S5). Despite photosynthetic C fixation during daytime, the total cumulative CO_2 flux in the planted treatments was higher than that of the cumulative CO_2 flux from the unplanted treatments ($p < 0.001$; Table S5).

3.2. CO_2 flux for dry-wet events

For the planted treatment, during the drought period, the daytime CO_2 flux was similar to the control treatment (Table S2), but the night-time CO_2 flux ($p < 0.001$; Fig. S3A; Table S3) and total daily CO_2 flux ($p < 0.001$; Fig. 2A; Table S4) decreased over time. The night-time CO_2 flux (Fig. S3B, $R^2 = 0.79$) and total daily CO_2 flux (Fig. 2B, $R^2 = 0.82$) were negatively correlated with water loss during the drought period. At the end of the experiment on day 138, the cumulative CO_2 flux of night-time and total after the dry-wet event was lower (19% and 22%, respectively) than the control ($p < 0.01$ and $p < 0.05$, respectively; Fig. 1), while the daytime CO_2 flux remained similar ($p > 0.05$). In contrast, for the unplanted soil, there was no change in the daytime, night-time, or total

258 CO_2 flux during the drought period ($p > 0.05$) in comparison to the control. Overall, the
259 CO_2 flux of daytime, night-time, and total were not influenced by soil dry-wet events
260 when plants were not present.
261
262 *3.3. CO_2 flux pulse during thawing period*
263 The daytime CO_2 flux from the planted treatments frozen to either -5 °C or -
264 10 °C increased on the first day after thawing ($p < 0.05$ and $p < 0.001$, respectively; Fig.
265 1; Table S2), compared to the flux under freezing conditions. The daytime CO_2 flux for
266 the planted treatment after the -10 °C freeze-thaw event continuously increased
267 throughout the thawing period. The night-time CO_2 flux and total daily flux also increased
268 rapidly on the first day of transition from freezing to thaw conditions at both freezing
269 temperatures ($p < 0.01$~0.001; Table S3 and S4). For the unplanted soil frozen to -10 °C,
270 the CO_2 flux during daytime, night-time, and total daily fluxes increased after the freeze-
271 thaw event ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively). Immediately after the -5 °C
272 freeze-thaw event, the daytime and the total CO_2 flux from unplanted soil increased ($p <$
273 0.05), whilst the night-time CO_2 flux were similar to the control ($p > 0.05$).
274
275 *3.4. Cumulative CO_2 fluxes after freeze-thaw events*
276 At the end of the experiment (day 138) in the planted treatment, the cumulative
277 daytime CO_2 flux after the -10 °C freeze-thaw event was higher (Fig. 1, $p < 0.01$), but
278 unchanged following freezing to -5 °C. The night-time and total daily CO_2 flux was lower
279 (115.6 and 78.6 g CO_2 m^{-2}, respectively) after freezing to -5 °C (19% and 20%,
280 respectively) and -10 °C (35% and 29%, respectively) compared with the unfrozen
281 control treatment ($p < 0.001 \sim 0.05$). On the other hand, there was no statistical difference

282 in the cumulative daytime, night-time, or total CO_2 flux from the freeze-thaw and control
283 unplanted soil treatments ($p > 0.05$).
284
285 *3.5. CH_4 flux*
286 There was no significant difference in CH_4 flux both from the planted and
287 unplanted soil treatments before or after a freeze-thaw or dry-wet event (Fig. S4; Table
288 S6).
289
290 *3.6. N_2O flux pulse after freeze-thaw events*
291 There was a significant increase in N_2O flux from the unplanted soil after
292 thawing from -10 °C ($p < 0.05$; Fig. 3; Table S7). N_2O peaked (97% over the control) on
293 the third day after thawing and then decreased for the duration of the experiment. Apart
294 from this, there was no change in N_2O emission from either planted or unplanted soil after
295 either thawing or rewetting events.
296
297 *3.7. Solute dynamics in soil*
298 No clear trend in soil DOC concentration was apparent with freeze-thaw or dry-
299 wet events in either planted or unplanted soils (Fig. 3; Table S8). As expected, the
300 presence of roots led to the depletion of nutrients from soil during the stress-free period
301 (e.g. NO_3^-, P, Ca, K, Mg), resulting in lower concentrations in the planted mesocosms
302 (Tables S11-S15). In the absence of plants, NO_3^-, Ca, K, Mg all gradually increased over
303 the experimental period irrespective of treatment. Similarly, after the most severe freeze-
304 thaw event (-10 °C), TDN, NO_3^-, Ca and Mg in soil gradually increased. NH_4^+, K and Na
305 remained similar to the control ($p > 0.05$; Figs. 3 and S5; Tables S9-S16). However, there

306 was little or no change in nutrient availability in soil after dry-wet or the milder freeze-
307 thaw event (-5 °C).
308
309 *3.8. Nutrient content of plants*
310 C and N content in shoots and roots in response to a dry-wet and freeze-thaw
311 cycle are shown in Figure S6. The C content in both shoots and roots was significantly
312 lower in comparison to other treatments after the -10 °C freeze-thaw event ($p < 0.001$),
313 although the effect on shoots was very minor. In contrast, N content in roots was
314 significantly increased after freezing to -10 °C ($p < 0.001$). However, the N content in
315 shoots was similar between treatments. Overall, macro and micro nutrients in roots tended
316 to be greater than other treatments including the control ($p < 0.001$, Fig. S7) after the -
317 10 °C freeze-thaw event, but this trend was not seen in the shoots (Fig. S8). All treatments
318 led to a reduction in shoot P concentration, but this was only seen in roots following the
319 -5 °C freeze-thaw event. Zn increased in roots after the dry-wet event and both this stress
320 and the mild freezing event reduced shoot Mn.
321
322 *3.9. Microbial community structure*
323 From PLFA measurements, the amounts of putative AM fungi, saprophytic fungi,
324 Gram-negative bacteria, eukaryotes, Gram-positive bacteria, actinomycetes in the planted
325 soil treatments were significantly greater than in the unplanted soil ($p < 0.001$; Table 1).
326 Principal component analysis of the PLFA data indicated a clear separation between
327 planted and unplanted soil ($R^2 = 0.67$, $p < 0.001$, Fig. S9). PC1 explained 95.7% of the
328 variance and PC2 explained 2.8 %.
329 The amounts of saprophytic fungi and Gram-negative bacteria in planted soil

were greater after the severe freeze-thaw (-10 °C) event than other treatments or the control ($p < 0.05$, Table 1). Principal component analysis of the PLFA data (PC1 variance explained 51.4 %, PC2 variance explained 27.2 %, Fig. 5) from planted soil after freeze-thaw events (-10 °C) separated the -10 °C freeze-thaw in planted soil group from all other groups ($R^2 = 0.63$, $p < 0.01$) due to an increase in night-time CO_2 flux ($R^2 = 0.83$, $p < 0.001$), total CO_2 flux ($R^2 = 0.63$, $p < 0.01$), TDN ($R^2 = 0.73$, $p < 0.001$), and NO_3^- ($R^2 = 0.56$, $p < 0.01$).

4. Discussion

4.1. Drying and re-wetting

Drying and re-wetting of soils had a surprisingly limited effect. Unplanted soils appeared to be completely unaffected, with fluxes of CO_2, CH_4, N_2O, soil solute concentrations and soil microbial community structure all remaining unchanged relative to controls. In partial contrast, the drying and re-wetting event reduced the total CO_2 efflux from planted mesocosms over the experimental period, although all other measured factors remained unchanged. The reduction in the CO_2 efflux appears to have been entirely caused by a reduction in the respiratory flux over the drying period, which correlated with the intensity of the drought. Following re-wetting, respiration rapidly returned to levels seen in the controls. The lack of an effect on respiration from unplanted soils, or any other effects on measured soil characteristics, suggests that the observed CO_2 effect was plant-mediated. Although net plant C fixation was maintained, the fact that respiration was reduced indicates that gross C fixation was also reduced during the drying period. However, as root respiration and root exudation are tightly coupled to recent photosynthesis with rapid turnover times for labile C compounds in the rhizosphere, it is

probable that daytime respiration rates exceeded those during night-time (Dilkes et al., 2001; Kuzyakov and Cheng 2001; Hill et al., 2008). Similarly, respiration and exudation from arbuscular mycorrhizas, for which the biomarker was higher in planted than unplanted soils is also likely to have been tightly coupled to recent C fixation (Jones et al., 2009). Thus, it seems likely that drought-driven reductions in respiratory substrate from photosynthesis were responsible for most, if not all, measured effects of the drought on CO_2 fluxes.

4.2. Mild freezing and thawing

As for the dry-wet event, the mild (-5 °C) freeze-thaw event had only a modest effect with soil solute concentrations, CH_4, N_2O and soil microbial community composition all unaffected whether plants were present or not. The overall drop in night-time and total CO_2 efflux from planted mesocosms with a maintenance of daytime net photosynthesis again suggests that photosynthetic capacity was reduced, and that this reduction in available substrate led to the reduction in respiration. The pulse of CO_2 released following thawing of planted mesocosms from -5 °C may suggest some damage to root tissues or microbes with rapid uptake and mineralisation of released C by surviving microbes (Schimel et al., 2007). Alternatively, it may indicate the post-stress metabolism of plant or microbial osmolytes accumulated during the stress, soil aggregate disruption or simply that respiration continued during freezing but accumulated CO_2 was not able to escape under frozen conditions (Cushman 2001; Lee et al., 2010; Wang et al., 2012). However, the lack of an effect in unplanted soils, again points to a plant mediated effect, with either direct damage to plant tissues or a magnified effect on soil microbes due to the increased C supply when plants were present.

4.3. Severe freezing and thawing

In contrast to both the other stresses, it is clear that freezing to -10 °C had a much more profound effect. The removal of C fixation during the light period and the loss of C from plant shoots and roots suggests that both photosynthetic tissues and roots were severely damaged by the -10 °C freeze-thaw event. The continuation of positive CO_2 fluxes during the remainder of the experimental period indicates that plant recovery from the stress was limited. Macronutrient and micronutrient concentrations, including N were increased in roots, probably due to a lack of mobility relative to the soluble C compounds lost during root damage by intracellular ice crystals (Borochov et al., 1987; Uemura and Steponku 1997). Soil DOC concentrations were not correspondingly increased, but this probably indicates rapid uptake and metabolism of released C by soil microbes (Hill et al., 2008; Schimel et al., 2007). The greater production of CO_2 during the daytime, night-time, and day/night combined flux, from both planted and unplanted mesocosms on the first day after thawing, supports this conclusion. Similarly, the increase in Gram-negative bacteria and fungal saprotrophs following the stress event probably results from this increase in available plant C (Balasooriya et al., 2014; Fanin et al., 2019).

Mg^{2+}, Ca^{2+}, and NO_3^- (and TDN) accumulated in the soil under the plants subjected to the most severe freezing, due probably to reduced plant uptake rather than a change in microbial activity. This further suggests greater damage to plants than under the other two stresses where these nutrients did not accumulate. Where plants were present, there was no change to N_2O efflux due to the accumulation of NO_3^- nor the release of labile C, due probably to their separation in time (Del Grosso et al., 2000). However, in unplanted soils where NO_3^- had already accumulated, the pulse of both N_2O

and CO_2 following thawing suggests that there was significant damage to microbial cells, which supplied available C substrate for both respiration and denitrification (Del Grosso et al., 2000; Skogland et al., 1998).

4.4. Limitations of the study

We note that our experiments were carried out under laboratory conditions and that these cannot truly reflect field conditions. For example, the constrained depth of the soil in the mesocosms prevented deep rooting and thus the potential to take up water from the subsoil to alleviate water stress. In addition, we did not apply a simultaneous thermal stress which is typically co-associated with periods of low rainfall. We also chose to use a wheat cultivar commonly used within the region. Further work could focus on cultivars with known greater freezing or drought tolerance or other crops (Schmidt, 1983; Zheng et a., 2018). The pattern of freezing was also slightly different in our soil to that in the field where thermal buffering from the subsoil may occur and where freezing commences at the soil surface and creates a freezing gradient down the soil profile. In addition, deeper roots in the subsoil would not suffer freezing injury. There is therefore a need to undertake similar experiments under field conditions.

5. Conclusions

Overall, although all stresses had some impact on planted soils, we found that the moderate drought event and the mild freeze-thaw event had fairly minor impacts on this simulated agroecosystem. Only the severe freeze-thaw event had any major impact on soil functioning. Further, it is clear that it was the effects on plants, which drove most of the measured effects, and only in the severe freezing event were free-living soil

426 microbes directly affected. The presence of plants gave rise to greater losses of CO_2 to
427 the atmosphere following the freezing events but, although some priming of soil C may
428 have taken place, the released CO_2 almost certainly resulted from the extra C available
429 due to previous C fixation by the plants (Kuzyakov, 2006). The only measured net
430 increase in GHG emissions occurred after the severe freezing event where no plants were
431 present. Consequently, the apparent magnitude of the short-term effects of extreme
432 weather events may be increased where winter plant cover, such as winter wheat, is
433 maintained in temperate agricultural soils. However, our results suggest that the overall
434 impact on future climate will be reduced relative to soils left unvegetated, even during
435 fairly severe freezing events.
436
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440
441 **References**
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