

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

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1	Role of plants in determining the soil response to either a single freeze-
2	thaw or dry-wet event
3	

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- 13

14 Abstract

15In a changing climate, extreme weather events are predicted to increase in frequency and 16 magnitude. These events may induce stress in plants and soil microbial communities, but 17the impact of climate extremes on root-soil interactions remains poorly understood. To better understand the response of a temperate agroecosystem to winter freezing and 18 19drought, a mild $(-5^{\circ}C)$ or severe $(-10^{\circ}C)$ freeze-thaw, or dry-wet cycle was imposed on 20mesocosms planted with winter wheat (Triticum aestivum L.) and unplanted soil. We measured the effect of the stresses on greenhouse gas (GHG) fluxes (CO₂, N₂O, CH₄), 21plant tissue composition, soil solute concentrations and soil microbial community 2223structure. Only the most severe freezing event had a direct effect on soils, with pulses of CO₂ and N₂O released after thawing. In contrast, all stresses reduced C fixation and 24respiration in planted treatments. Total CO₂ flux from planted mesocosms was reduced 25during the drought period and CO₂ flux was negatively correlated with soil water content. 2627The severe freeze-thaw event caused lasting damage to plants and increased 28rhizodeposition, resulting in increased CO₂ efflux and a small alteration in soil microbial community composition. The presence of plants resulted in significantly greater total CO₂ 2930 flux following freeze-thaw or dry-wet events, but only in unplanted soil was there a net 31increase in GHG emissions. These results demonstrate that, although the effects of stress 32appear magnified where plants are present, the maintenance of winter plant cover in 33 temperate agricultural soils reduces the effects of extreme weather events on future 34 climate.

35

36 Keywords: Climate change, carbon cycle, sustainable agriculture, nitrogen cycle,

- 37 nutrient dynamics.
- 38

39 **Declarations of Interest**

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

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43 **1. Introduction**

Climate change is likely to alter the frequency of freeze-thaw or dry-wet events 44 with wide-reaching implications for the functioning of temperate agroecosystems 45 46(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., 472013; Yang and Wang 2019), and can trigger stress responses in both plants (Harp et al., 482010; Skinner, 2015) and soil microbes (Schimel et al., 2007). Freezing and drying 49 processes can damage soil microbial and root cells, releasing organic carbon (C) and 50nitrogen (N), which results in an increase in soil solution concentrations (Schimel et al., 51522007). These changes in soil C and N concentration may directly affect native soil organic 53matter (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 54events on soil gas fluxes have been widely reported (Kim et al., 2012), with an increase 5556in CO₂, N₂O, and CH₄ 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 57magnitude of freeze-thaw or dry-wet events may therefore have important implications 58for the net GHG budget of agroecosystems. 59

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

72 CO_2 release from soil is due to a combination of heterotrophic (microbial) and 73autotrophic (plant) respiration (Wang et al., 2014). Although separating heterotrophic and 74autotrophic respiration may help to understand C cycling under extreme environmental change (Casals et al., 2011; Zhou et al., 2009), root-soil interactions under these 7576 conditions are rarely characterized. In mesocosms, drought has been shown to increase 77root respiration in wheat, although the responses are cultivar specific (Liu et al., 2004). 78However, comparatively little is known about cereal root gas exchange in response to low freezing temperatures. In the field, catching natural freeze-thaw events in temperate 7980 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.

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

94

95 2. Materials and methods

96 2.1. Sample collection

Soil samples (5-10 cm depth, Ah horizon) under Lolium perenne L. were 97 98 collected from a crumb-structured Eutric Cambisol in a sheep-grazed grassland located at the Henfaes Experimental Station at Abergwyngregyn, Gwynedd, North Wales 99 (53°14'N, 04°01'W). The soil characteristics for this site is shown in Table S1. The mean 100 annual air temperature at the site is 10.6 °C (max 28.6 °C, min -7.6 °C) and the mean 101 annual rainfall is 1055 mm y^{-1} . The lowest temperature ever recorded at the site was 102-23 °C in 1940. Fertilizer is regularly added to the grassland (50 kg N ha⁻¹, 10 kg P ha⁻¹, 103 10 kg K ha⁻¹). Prior to use, the soil was sieved to pass 1 cm and any roots and stones 104 removed. Previous experiments in this soil have shown that this large sieve size 105minimizes changes to soil structure and microbial functioning (Jones and Willett, 2006). 106 pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-distilled water 107 108 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. 109

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111 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), totaling 32 containers. All containers were transferred to a climate-controlled plant growth cabinet (CMP6010; Conviron Inc., Winnipeg, Canada) maintained with a 8 h photoperiod, light intensity of 180 μ mol m⁻² s⁻¹, and relative humidity of 75 % during the experiment. Soil moisture was maintained at 20-25 % of field capacity by adding distilled 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.

124The experiment consisted of two main treatments 1) a single freeze-thaw event, or 2) a single dry-wet event. For the freeze-thaw treatments, after a 5-week pre-treatment 125period, all samples were transferred to incubators at either -5 °C or -10 °C for 24 h to 126127 simulate a freezing period. They were then placed back into a growth cabinet at 10 °C and allowed to thaw naturally. The temperature of -5 °C was considered to be realistic for 128typical winter conditions in the UK. An extreme freezing treatment of -10 °C was used to 129reflect conditions which occur infrequently, but also because winter wheat plants have a 130high degree of freezing tolerance, being able to survive from -4 to -15 °C (Gusta et al., 1311997). 132

For the dry-wet treatment, after a week-long pre-treatment period (10 °C), the samples were maintained in a winter dry condition by natural-drying (not adding water) in open containers placed in the growth cabinet (10 °C) for 5 weeks. This reflects periods of spring drought recently experienced in the region. Water loss was determined by 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 138(at the end of drought period) and distilled water was added to the soil surface to bring 139140the water content back to the original water content. Overall, the experiment had 8 141treatments: 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 / 142+10 °C) with plants, 5) Control without plants (+10 °C, constant soil moisture level), 6) 143Dry-wet without plants (+10 °C), 7) Freeze-thaw (-5 °C / +10 °C)) without plants, 8) 144Freeze-thaw (-10 $^{\circ}C$ / +10 $^{\circ}C$) without plants. 145

- 146
- 147 2.3. Soil greenhouse gas emissions

148Greenhouse gas (GHG) samples were taken from the experimental containers (including entire plants, roots and soil). At each sampling time, gas-tight lids containing 149a Suba-seal[®] gas sampling port (Sigma-Aldrich Ltd, Poole, UK) were placed on the 150151polypropylene containers. 20 ml gas samples were then taken from the containers' 152headspace 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. 153154Preliminary checks where samples were taken every 15 mins showed that the production of CO2 and N2O from the mesocosms was linear over time. On each occasion, gas 155156sampling took place twice in the same day at daytime (light condition) and night (dark 157condition). CO₂, N₂O, and CH₄ concentrations in the vials were determined by gas chromatography using a Clarus 500 GC with a Turbomatrix (HS-40) autoanalyzer 158(PerkinElmer Inc., Waltham, MA). CO₂ and CH₄ were detected by a flame ionization 159detector (FID) and N₂O by a ⁶³Ni electron capture detector (ECD). The gas flux was 160calculated according to Dunn et al. (2016), within the linear portion of a standard curve. 161

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.

165

166 2.4. Soil chemical analysis

167 Soil nutrient availability was estimated according to Jones and Willett (2006). 168 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 169 Buhler GmbH, SM-30, Germany; 200 rev min⁻¹). After shaking for 10 min, extracts were 170171centrifuged (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 172dissolved N (TDN) in soil solution were measured using a Multi N/C 2100/2100 analyzer 173(AnalytikJena AG, Jena, Germany). Anions and cations were measured by ion 174175chromatography analysis using an 850 Professional IC (Metrohm Ltd., Runcorn, UK).

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177 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).

185

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 (2012). Briefly, samples (2 g) from each container were freeze-dried and Bligh-Dyer 189 extractant (4.0 ml) containing internal standard was added. Tubes were sonicated in an 190191 ultrasonic cleaning bath (10 min, room temperature) before rotating end-over-end (2 h). 192After centrifuging (10 min) the liquid phase was transferred to clean 13 mm \times 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water were added. The upper 193phase was removed by aspiration and discarded while the lower phase, containing the 194extracted lipids, was evaporated (30 °C). Lipid classes were separated by solid phase 195extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well 196 (Phenomenex, Torrance, CA, USA). Phospholipids were eluted with 0.5 ml of 5:5:1 197 methanol:chloroform:water (Findlay, 2004) into 1.5 ml glass vials (Multi-Tier microplate, 198199 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 PTFE/silicon cap mat (E&K Scientific, Santa Clara, CA, USA), and incubated (37 °C, 15 201202min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were added. The chloroform 203was 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 205Technologies, Wilmington, DE, USA) equipped with autosampler, split-splitless inlet, 206and flame ionization detector. Fatty acid methyl esters (FAMEs) were separated on an Agilent Ultra 2 column, 25 m long \times 0.2 mm internal diameter \times 0.33 µm film thickness. 207Standard nomenclature was followed for PLFA (Frostegård et al., 1993). Taxonomic 208groups were ascribed to individual PLFAs using the Sherlock[®] PLFA Method and Tools 209

Package (PLFAD1; Microbial ID Inc., Newark, DE). Over one hundred fatty acids were 210identified in the soil samples, however, we only present results from the twenty-nine 211whose concentration was higher than 0.5% of the total PLFAs, classified per taxonomic 212213group. 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 ω 9c were used for 214Gram+ bacteria; 16:1 \omega 5c, 16:1 \omega7c, 16:1 \omega9c, 17:1 \omega8c, 17:0 cyclo \omega7c, 18:1 \omega5c, 18:1 215216ω7c, 18:1 ω9c and 19:0 cyclo ω7c were used for Gram- bacteria; 16:0 10 methyl, 17:1 ω 7c 10 methyl, 18:0 10 methyl and 18:1 ω 7c 10 methyl for actinomycetes; 18:2 ω 6c for 217saprotrophic fungi; and 16:1 w5c as biomarker for putative arbuscular mycorrhizal fungi 218219(Sánchez-Rodríguez et al., 2019).

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221 2.7. Statistical analysis

Effects of plant (with plant vs without plant) was analyzed using two-way 222223analysis of variance (ANOVA) without interactions. Tukey post-hoc test was used to 224compare treatment means. Pearson's product-moment correlation was carried out between factors to explore relationship between water contents and GHG flux. One-way ANOVA 225226was 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 227228(Frostegård et al., 1993) were analyzed by principal component analysis (PCA). Significance was evaluated by permutational multivariate analysis of variance 229(PERMANOVA) with 999 permutations. Statistical analyses were performed using R 230v.4.1.0 project (R Development Core Team, 2020) in the 'vegan' package (Oksanen et al., 2312020). 232

233

3. Results

235 *3.1. CO*² *flux for planted versus non-planted soil*

Absolute and cumulative CO₂ fluxes of daytime, night-time and daily totals are 236237shown in Figure 1 (positive fluxes indicate CO₂ efflux i.e. respiration; negative fluxes indicate influx i.e. CO₂ fixation). Due to photosynthetic C fixation, the daytime 238cumulative CO₂ flux from the planted treatments was generally lower than the flux from 239240unplanted, regardless of specific treatment type (p < 0.001; Table S5). In comparison, in both the planted and unplanted treatments the cumulative CO₂ flux during the longer (16 241h) night-time period was higher than in the daytime, but the cumulative CO₂ flux from 242planted treatments was higher than the unplanted soil (p < 0.001; Table S5). Despite 243photosynthetic C fixation during daytime, the total cumulative CO₂ flux in the planted 244treatments was higher than that of the cumulative CO₂ flux from the unplanted treatments 245(*p* < 0.001; Table S5). 246

247

248 3.2. CO₂ flux for dry-wet events

For the planted treatment, during the drought period, the daytime CO_2 flux was 249250similar to the control treatment (Table S2), but the night-time CO₂ flux (p < 0.001; Fig. S3A; Table S3) and total daily CO₂ flux (p < 0.001; Fig. 2A; Table S4) decreased over 251time. The night-time CO₂ flux (Fig. S3B, $R^2 = 0.79$) and total daily CO₂ flux (Fig. 2B, R^2 252= 0.82) were negatively correlated with water loss during the drought period. At the end 253254of the experiment on day 138, the cumulative CO₂ 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 < 0.012550.05, respectively; Fig. 1), while the daytime CO₂ flux remained similar (p > 0.05). In 256contrast, for the unplanted soil, there was no change in the daytime, night-time, or total 257

258 CO₂ flux during the drought period (p > 0.05) in comparison to the control. Overall, the 259 CO₂ 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*

The daytime CO₂ flux from the planted treatments frozen to either -5 °C or -26326410 °C increased on the first day after thawing (p < 0.05 and p < 0.001, respectively; Fig. 1; Table S2), compared to the flux under freezing conditions. The daytime CO₂ flux for 265the planted treatment after the -10 °C freeze-thaw event continuously increased 266throughout the thawing period. The night-time CO₂ flux and total daily flux also increased 267rapidly on the first day of transition from freezing to thaw conditions at both freezing 268269temperatures ($p < 0.01 \sim 0.001$; Table S3 and S4). For the unplanted soil frozen to -10 °C, the CO₂ flux during daytime, night-time, and total daily fluxes increased after the freeze-270271thaw event (p < 0.001, p < 0.05, and p < 0.001, respectively). Immediately after the -5 °C freeze-thaw event, the daytime and the total CO_2 flux from unplanted soil increased (p < p2720.05), whilst the night-time CO₂ flux were similar to the control (p > 0.05). 273

274

275 *3.4. Cumulative CO₂ fluxes after freeze-thaw events*

At the end of the experiment (day 138) in the planted treatment, the cumulative daytime CO₂ flux after the -10 °C freeze-thaw event was higher (Fig. 1, p < 0.01), but unchanged following freezing to -5 °C. The night-time and total daily CO₂ flux was lower (115.6 and 78.6 g CO₂ m⁻², respectively) after freezing to -5 °C (19% and 20%, respectively) and -10 °C (35% and 29%, respectively) compared with the unfrozen control treatment ($p < 0.001 \sim 0.05$). On the other hand, there was no statistical difference in the cumulative daytime, night-time, or total CO₂ flux from the freeze-thaw and control unplanted soil treatments (p > 0.05).

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285 3.5. CH<sub>4</sub> flux
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There was no significant difference in CH₄ flux both from the planted and unplanted soil treatments before or after a freeze-thaw or dry-wet event (Fig. S4; Table S6).

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290 *3.6. N*₂*O flux pulse after freeze-thaw events*

There was a significant increase in N₂O flux from the unplanted soil after thawing from -10 °C (p < 0.05; Fig. 3; Table S7). N₂O peaked (97% over the control) on the third day after thawing and then decreased for the duration of the experiment. Apart from this, there was no change in N₂O emission from either planted or unplanted soil after either thawing or rewetting events.

296

297 *3.7. Solute dynamics in soil*

298No clear trend in soil DOC concentration was apparent with freeze-thaw or dry-299wet 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 (e.g. NO₃⁻, P, Ca, K, Mg), resulting in lower concentrations in the planted mesocosms 301 302(Tables S11-S15). In the absence of plants, NO₃⁻, Ca, K, Mg all gradually increased over the experimental period irrespective of treatment. Similarly, after the most severe freeze-303 thaw event (-10 °C), TDN, NO₃⁻, Ca and Mg in soil gradually increased. NH₄⁺, K and Na 304 remained similar to the control (p > 0.05; Figs. 3 and S5; Tables S9-S16). However, there 305

was little or no change in nutrient availability in soil after dry-wet or the milder freezethaw event (-5 $^{\circ}$ C).

- 308
- 309 *3.8. Nutrient content of plants*

C and N content in shoots and roots in response to a dry-wet and freeze-thaw 310 cycle are shown in Figure S6. The C content in both shoots and roots was significantly 311lower in comparison to other treatments after the -10 °C freeze-thaw event (p < 0.001), 312although the effect on shoots was very minor. In contrast, N content in roots was 313 significantly increased after freezing to -10 °C (p < 0.001). However, the N content in 314315shoots was similar between treatments. Overall, macro and micro nutrients in roots tended to be greater than other treatments including the control (p < 0.001, Fig. S7) after the -31610 °C freeze-thaw event, but this trend was not seen in the shoots (Fig. S8). All treatments 317led to a reduction in shoot P concentration, but this was only seen in roots following the 318319 -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*

From PLFA measurements, the amounts of putative AM fungi, saprophytic fungi, Gram-negative bacteria, eukaryotes, Gram-positive bacteria, actinomycetes in the planted soil treatments were significantly greater than in the unplanted soil (p < 0.001; Table 1). Principal component analysis of the PLFA data indicated a clear separation between planted and unplanted soil ($R^2 = 0.67$, p < 0.001, Fig. S9). PC1 explained 95.7% of the 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 freezethaw 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 CO₂ flux ($R^2 = 0.83$, p <0.001), total CO₂ flux ($R^2 = 0.63$, p < 0.01), TDN ($R^2 = 0.73$, p < 0.001), and NO₃⁻ ($R^2 =$ 0.56, p < 0.01).

337

338 4. Discussion

339 4.1. Drying and re-wetting

340 Drying and re-wetting of soils had a surprisingly limited effect. Unplanted soils appeared to be completely unaffected, with fluxes of CO₂, CH₄, N₂O, soil solute 341concentrations and soil microbial community structure all remaining unchanged relative 342343to controls. In partial contrast, the drying and re-wetting event reduced the total CO_2 344efflux from planted mesocosms over the experimental period, although all other measured factors remained unchanged. The reduction in the CO₂ efflux appears to have been 345346entirely caused by a reduction in the respiratory flux over the drying period, which 347correlated with the intensity of the drought. Following re-wetting, respiration rapidly 348returned to levels seen in the controls. The lack of an effect on respiration from unplanted 349soils, or any other effects on measured soil characteristics, suggests that the observed CO₂ 350effect 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 351period. However, as root respiration and root exudation are tightly coupled to recent 352photosynthesis with rapid turnover times for labile C compounds in the rhizosphere, it is 353

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.

361

362 4.2. Mild freezing and thawing

As for the dry-wet event, the mild (-5 °C) freeze-thaw event had only a modest 363 364 effect with soil solute concentrations, CH₄, N₂O and soil microbial community composition all unaffected whether plants were present or not. The overall drop in night-365time and total CO₂ efflux from planted mesocosms with a maintenance of daytime net 366 photosynthesis again suggests that photosynthetic capacity was reduced, and that this 367 368 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 369 370 to root tissues or microbes with rapid uptake and mineralisation of released C by surviving 371microbes (Schimel et al., 2007). Alternatively, it may indicate the post-stress metabolism 372of plant or microbial osmolytes accumulated during the stress, soil aggregate disruption 373or simply that respiration continued during freezing but accumulated CO₂ was not able to 374escape 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, 375with either direct damage to plant tissues or a magnified effect on soil microbes due to 376 the increased C supply when plants were present. 377

378

379

4.3. Severe freezing and thawing

380 In contrast to both the other stresses, it is clear that freezing to -10 °C had a much 381 more profound effect. The removal of C fixation during the light period and the loss of C 382from 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 CO₂ 383 384 fluxes during the remainder of the experimental period indicates that plant recovery from the stress was limited. Macronutrient and micronutrient concentrations, including N were 385 386 increased in roots, probably due to a lack of mobility relative to the soluble C compounds 387 lost during root damage by intracellular ice crystals (Borochov et al., 1987; Uemura and 388 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 389 al., 2008; Schimel et al., 2007). The greater production of CO₂ during the daytime, night-390 391time, and day/night combined flux, from both planted and unplanted mesocosms on the 392 first day after thawing, supports this conclusion. Similarly, the increase in Gram-negative 393 bacteria and fungal saprotrophs following the stress event probably results from this 394increase 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 N₂O efflux due to the accumulation of NO₃⁻ nor the release of labile C, due probably to their separation in time (Del Grosso et al., 2000). However, in unplanted soils where NO₃⁻ had already accumulated, the pulse of both N₂O and CO₂ 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).

405

406 *4.4. Limitations of the study*

407 We note that our experiments were carried out under laboratory conditions and 408 that these cannot truly reflect field conditions. For example, the constrained depth of the 409 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 410 411 stress which is typically co-associated with periods of low rainfall. We also chose to use 412a wheat cultivar commonly used within the region. Further work could focus on cultivars 413 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 414 415field where thermal buffering from the subsoil may occur and where freezing commences 416 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 417418 similar experiments under field conditions.

419

420 **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

microbes directly affected. The presence of plants gave rise to greater losses of CO₂ to 426the atmosphere following the freezing events but, although some priming of soil C may 427428have taken place, the released CO₂ almost certainly resulted from the extra C available 429due to previous C fixation by the plants (Kuzyakov, 2006). The only measured net increase in GHG emissions occurred after the severe freezing event where no plants were 430 431present. Consequently, the apparent magnitude of the short-term effects of extreme 432weather events may be increased where winter plant cover, such as winter wheat, is maintained in temperate agricultural soils. However, our results suggest that the overall 433impact on future climate will be reduced relative to soils left unvegetated, even during 434435fairly severe freezing events.

436

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