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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

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.

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.

42

43 ## 1. Introduction

44 Climate change is likely to alter the frequency of freeze-thaw or dry-wet events
45 with wide-reaching implications for the functioning of temperate agroecosystems
46 (Kreyling et al., 2007; Donat et al., 2016). Freeze-thaw or dry-wet events are important
47 ecological processes that affect soil hydrology and thermodynamics (Harrison-Kirk et al.,
48 2013; Yang and Wang 2019), and can trigger stress responses in both plants (Harp et al.,
49 2010; Skinner, 2015) and soil microbes (Schimel et al., 2007). Freezing and drying
50 processes can damage soil microbial and root cells, releasing organic carbon (C) and
51 nitrogen (N), which results in an increase in soil solution concentrations (Schimel et al.,
52 2007). These changes in soil C and N concentration may directly affect native soil organic
53 matter (SOM) turnover rates, which may stimulate greenhouse gas (GHG) emissions
54 (Kim et al., 2012). Field and laboratory studies of the effects of freeze-thaw or dry-wet
55 events on soil gas fluxes have been widely reported (Kim et al., 2012), with an increase
56 in CO_2, N_2O, and CH_4 following thawing or rewetting, commonly observed (Xiang et al.,
57 2008; Wu et al., 2010 Kim et al., 2012). The predicted increase in the frequency and
58 magnitude of freeze-thaw or dry-wet events may therefore have important implications
59 for the net GHG budget of agroecosystems.

60 The root system plays an important role in the response of the soil environment
61 to freeze-thaw or dry-wet events (Whitmore and Whalley 2009; Hosokawa et al., 2017).
62 Root exudates are primarily derived from recently assimilated C and represent a semi-
63 continuous input of labile C to soil (Jones et al., 2009). Most exudates consist of sugars,
64 amino acids, and organic acids (Badri and Vivanco, 2009; Carvalhais et al., 2011) and
65 these low molecular weight (MW) organic compounds can be released into soil by passive
66 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

91 effects of freeze-thaw or dry-wet events on GHG fluxes from planted soil would be
92 greater than from unplanted soil due to the release of root-derived C. The conditions used
93 were chosen to reflect soil conditions in autumn versus spring sown cereal crops.
94

95 **2. Materials and methods**

96 *2.1. Sample collection*

97 Soil samples (5-10 cm depth, Ah horizon) under *Lolium perenne* L. were
98 collected from a crumb-structured Eutric Cambisol in a sheep-grazed grassland located
99 at the Henfaes Experimental Station at Abergwyngregyn, Gwynedd, North Wales
100 (53°14'N, 04°01'W). The soil characteristics for this site is shown in Table S1. The mean
101 annual air temperature at the site is 10.6 °C (max 28.6 °C, min −7.6 °C) and the mean
102 annual rainfall is 1055 mm y^{-1}. The lowest temperature ever recorded at the site was
103 −23 °C in 1940. Fertilizer is regularly added to the grassland (50 kg N ha^{-1}, 10 kg P ha^{-1},
104 10 kg K ha^{-1}). Prior to use, the soil was sieved to pass 1 cm and any roots and stones
105 removed. Previous experiments in this soil have shown that this large sieve size
106 minimizes changes to soil structure and microbial functioning (Jones and Willett, 2006).
107 pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-distilled water
108 extract (Smith and Doran 1996). Soil moisture and organic matter content were
109 determined by mass loss following heating for 24 h at 105 °C and 450 °C, respectively.
110

111 *2.2. Experimental design*

112 Four replicates of field moist soil (700 g) were placed into polypropylene
113 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

138 50 - 60 % of field capacity. For rewetting of soil, the amount of water lost was calculated
139 (at the end of drought period) and distilled water was added to the soil surface to bring
140 the water content back to the original water content. Overall, the experiment had 8
141 treatments: 1) Control with plants (+10 °C, constant soil moisture level), 2) Dry-wet with
142 plants (+10 °C), 3) Freeze -thaw (-5 °C / +10 °C) with plants, 4) Freeze-thaw (-10 °C /
143 +10 °C) with plants, 5) Control without plants (+10 °C, constant soil moisture level), 6)
144 Dry-wet without plants (+10 °C), 7) Freeze-thaw (-5 °C / +10 °C)) without plants, 8)
145 Freeze-thaw (-10 °C / +10 °C) without plants.

146

147 *2.3. Soil greenhouse gas emissions*

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

162 Daily cumulative flux was calculated assuming constant flux between two measurements,
163 multiplying hourly flux by the number of hours (8 during light / 16 during dark for
164 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
169 containers and extracted in 10 ml deionized water on a reciprocating shaker (Edmund
170 Buhler GmbH, SM-30, Germany; 200 rev min^{-1}). After shaking for 10 min, extracts were
171 centrifuged (6850 *g*, 5 min) and then filtered through PES 0.45 μm syringe filters (Triple
172 Red; Avidity Science Ltd, Long Crendon, UK). Soil dissolved organic C (DOC) and total
173 dissolved N (TDN) in soil solution were measured using a Multi N/C 2100/2100 analyzer
174 (AnalytikJena AG, Jena, Germany). Anions and cations were measured by ion
175 chromatography analysis using an 850 Professional IC (Metrohm Ltd., Runcorn, UK).

176

177 *2.5. Plant analysis*

178 At the end of the experiment on day 138, the plants were removed from the soil,
179 separated into shoots and roots, and oven-dried at 60 °C for 72 h. The shoots and the roots
180 were then ground to a fine powder using a MM200 ball mill (Retsch GmbH, Düsseldorf,
181 Germany). Total C and N of shoots and roots tissues were determined using a Truspec
182 CN analyzer (Leco Corp., St Joseph, MI, USA). Major and trace elements in shoots and
183 roots were determined using a S2 Picofox, Total Reflection X-ray Fluorescence (TXRF)
184 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
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

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

220

221 *2.7. Statistical analysis*

222 Effects of plant (with plant vs without plant) was analyzed using two-way
223 analysis of variance (ANOVA) without interactions. Tukey post-hoc test was used to
224 compare treatment means. Pearson's product-moment correlation was carried out between
225 factors to explore relationship between water contents and GHG flux. One-way ANOVA
226 was performed to determine the effect of freeze-thaw or dry-wet events on plant and root
227 chemicals followed by Tukey post-hoc test. Normalized fatty acid PLFA profiles
228 (Frostegård et al., 1993) were analyzed by principal component analysis (PCA).
229 Significance was evaluated by permutational multivariate analysis of variance
230 (PERMANOVA) with 999 permutations. Statistical analyses were performed using R
231 v.4.1.0 project (R Development Core Team, 2020) in the 'vegan' package (Oksanen et al.,
232 2020).

233

234 **3. Results**

235 *3.1. CO_2 flux for planted versus non-planted soil*

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

247

248 *3.2. CO_2 flux for dry-wet events*

249 For the planted treatment, during the drought period, the daytime CO_2 flux was
250 similar to the control treatment (Table S2), but the night-time CO_2 flux ($p < 0.001$; Fig.
251 S3A; Table S3) and total daily CO_2 flux ($p < 0.001$; Fig. 2A; Table S4) decreased over
252 time. The night-time CO_2 flux (Fig. S3B, $R^2 = 0.79$) and total daily CO_2 flux (Fig. 2B, R^2
253 $= 0.82$) were negatively correlated with water loss during the drought period. At the end
254 of the experiment on day 138, the cumulative CO_2 flux of night-time and total after the
255 dry-wet event was lower (19% and 22%, respectively) than the control ($p < 0.01$ and $p <$
256 0.05, respectively; Fig. 1), while the daytime CO_2 flux remained similar ($p > 0.05$). In
257 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

330 were greater after the severe freeze-thaw (-10 °C) event than other treatments or the
331 control ($p < 0.05$, Table 1). Principal component analysis of the PLFA data (PC1 variance
332 explained 51.4 %, PC2 variance explained 27.2 %, Fig. 5) from planted soil after freeze-
333 thaw events (-10 °C) separated the -10 °C freeze-thaw in planted soil group from all other
334 groups ($R^2 = 0.63$, $p < 0.01$) due to an increase in night-time CO_2 flux ($R^2 = 0.83$, $p <$
335 0.001), total CO_2 flux ($R^2 = 0.63$, $p < 0.01$), TDN ($R^2 = 0.73$, $p < 0.001$), and NO_3^- ($R^2 =$
336 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
341 appeared to be completely unaffected, with fluxes of CO_2, CH_4, N_2O, soil solute
342 concentrations and soil microbial community structure all remaining unchanged relative
343 to controls. In partial contrast, the drying and re-wetting event reduced the total CO_2
344 efflux from planted mesocosms over the experimental period, although all other measured
345 factors remained unchanged. The reduction in the CO_2 efflux appears to have been
346 entirely caused by a reduction in the respiratory flux over the drying period, which
347 correlated with the intensity of the drought. Following re-wetting, respiration rapidly
348 returned to levels seen in the controls. The lack of an effect on respiration from unplanted
349 soils, or any other effects on measured soil characteristics, suggests that the observed CO_2
350 effect was plant-mediated. Although net plant C fixation was maintained, the fact that
351 respiration was reduced indicates that gross C fixation was also reduced during the drying
352 period. However, as root respiration and root exudation are tightly coupled to recent
353 photosynthesis with rapid turnover times for labile C compounds in the rhizosphere, it is

354 probable that daytime respiration rates exceeded those during night-time (Dilkes et al.,
355 2001; Kuzyakov and Cheng 2001; Hill et al., 2008). Similarly, respiration and exudation
356 from arbuscular mycorrhizas, for which the biomarker was higher in planted than
357 unplanted soils is also likely to have been tightly coupled to recent C fixation (Jones et
358 al., 2009). Thus, it seems likely that drought-driven reductions in respiratory substrate
359 from photosynthesis were responsible for most, if not all, measured effects of the drought
360 on CO_2 fluxes.

361

362 *4.2. Mild freezing and thawing*

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

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
382 from plant shoots and roots suggests that both photosynthetic tissues and roots were
383 severely damaged by the -10 °C freeze-thaw event. The continuation of positive CO_2
384 fluxes during the remainder of the experimental period indicates that plant recovery from
385 the stress was limited. Macronutrient and micronutrient concentrations, including N were
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
389 probably indicates rapid uptake and metabolism of released C by soil microbes (Hill et
390 al., 2008; Schimel et al., 2007). The greater production of CO_2 during the daytime, night-
391 time, 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
394 increase in available plant C (Balasooriya et al., 2014; Fanin et al., 2019).

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

402 and CO_2 following thawing suggests that there was significant damage to microbial cells,
403 which supplied available C substrate for both respiration and denitrification (Del Grosso
404 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
410 the subsoil to alleviate water stress. In addition, we did not apply a simultaneous thermal
411 stress which is typically co-associated with periods of low rainfall. We also chose to use
412 a 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
414 et a., 2018). The pattern of freezing was also slightly different in our soil to that in the
415 field 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
417 roots in the subsoil would not suffer freezing injury. There is therefore a need to undertake
418 similar experiments under field conditions.
419
420 **5. Conclusions**
421 Overall, although all stresses had some impact on planted soils, we found that
422 the moderate drought event and the mild freeze-thaw event had fairly minor impacts on
423 this simulated agroecosystem. Only the severe freeze-thaw event had any major impact
424 on soil functioning. Further, it is clear that it was the effects on plants, which drove most
425 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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