1 **Water stress history and wheat genotype modulate rhizosphere**
2 **microbial response to drought**

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4 Hamed Azarbad[1*], Philippe Constant[1], Charlotte Giard-Laliberté[1], Luke D. Bainard[2] and
5 Etienne Yergeau[1]

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7 [1]Centre INRS-Institut Armand-Frappier, Institut national de la recherche scientifique, Laval,
8 QC, Canada

9 [2]Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, 1
10 Airport Road, Swift Current, SK, S9H 3X2, Canada

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13 [*]Corresponding author at: Centre INRS-Institut Armand-Frappier, Institut national de la recherche
14 scientifique, Laval, QC, Canada. Tel.: +1 450-687-5010 #4053
15 E-mail address: Hamed.Azarbad@iaf.inrs.ca
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25 **Abstract**

26 Different crop genotypes and soils with different water stress histories are known to harbour
27 different microorganisms, but their relative effect in the response of plant-associated microbes
28 to water stress is not known. In a pot experiment, four wheat genotypes (two with recognized
29 drought resistance and two without) were grown in semi-arid soils with different irrigation
30 histories (irrigated and non-irrigated soils) and exposed to four levels of soil water content
31 (ranging from high to low water content). After one month of exposure to different soil water
32 content, we examined plant biomass as well as a general (CO_2 production) and a specialized
33 (soil uptake of atmospheric H_2) functional processes in the rhizosphere. We further measured
34 the abundance of bacteria and fungi in the rhizosphere using real-time PCR. Wheat shoot
35 biomass was lower when growing in non-irrigated soils under low water content. In contrast,
36 under moderate water contents wheat grown in non-irrigated soils had a significantly higher
37 root biomass compared with those grown in irrigated soils. CO_2 production did not differ
38 between genotypes and soil irrigation histories under low soil water content. However, we
39 found significantly higher H_2 oxidation rates under low water content in the rhizosphere of
40 plants growing in formerly irrigated soil as compared to those grown in formerly non-irrigated
41 soils, although the intensity of the change was genotype-specific. Bacterial abundance was
42 more sensitive to decreasing soil water content than fungal abundance and was mainly
43 influenced by soil water stress history. Taken together, our results highlight that wheat
44 breeding history and soil water stress history differentially influence crop growth performance,
45 a specialized and a general rhizosphere processes, and rhizosphere bacterial and fungal
46 abundance in the face of decreasing soil water content.

47

48 **Keywords:** Drought; Wheat; Functional process; Rhizosphere; 16S; ITS

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51 **1. Introduction**

52 Earth system models predict that some regions will experience strong changes in climate
53 variability with the potential for increases in extreme events (Malhi et al., 2008). One of the
54 major consequences of climate change will be increased frequency and severity of drought
55 (IPCC 2013). This is particularly true in western Canada, a critical area for food production,
56 where increasing the severity of drought is expected to dramatically reduce yield in crop plants
57 by up to 50% or more (Duggan et al., 2000; Bagci et al., 2007). It is therefore critical to increase
58 efforts to reduce the negative effects of water stress on crop production (Ngumbi and
59 Kloepper, 2016).

60

61 There are different approaches to improve plant adaptation in the face of abiotic stresses,
62 including traditional plant breeding, genetic engineering of crops and management of the
63 plant-associated microbiome (Fleury et al., 2010; Quiza et al., 2015). Plant breeding and
64 genetic engineering of crops can help plants better tolerate sub-optimal conditions (Coleman-
65 Derr and Tringe, 2014). However, many studies of stress-tolerant genotypes did not consider
66 biotic and abiotic aspects of the soil environment and microbial impact on plant stress
67 tolerance in response to stressors (Budak et al., 2013; Swamy and Kumar, 2013; Waterer et
68 al., 2010). It has been suggested that the plant-associated microbiome, which can either be
69 vertically (from parent plants to offspring) or horizontally (through plant uptake of microbes or
70 microbial uptake of genes fragments from the environment) transferred from one generation
71 to the other, may help plants to withstand stress conditions (Strobel, 2006). Several
72 mechanisms have been proposed to be involved in the enhancement of plant drought
73 tolerance by plant-associated microbiome, such as an increase in root biomass (Naseem and
74 Bano, 2014), deeper root system (Yasmin et al., 2013), modulation of plant drought stress
75 genes (Timmusk and Wagner, 1999) and suppression of ethylene emissions by 1-
76 aminocyclopropane-1-carboxylic acid (ACC) deaminase (Mayak et al., 2004).

77 Root-associated microorganisms could be directly affected by water stress and indirectly by
78 the plant response to stress (Sanaullah et al., 2011). It has been demonstrated that as plants
79 experience water stress the root biomass increase in compared with the shoot in order to
80 enhance nutrient and water uptake from the soil environment (Zang et al., 2014) and, as the
81 results, the quantity and quality of belowground C inputs change (Canarini and Dijkstra, 2015;
82 Fuchslueger et al 2014), which in turn affect the rhizosphere microbiome (Grayston et al.,
83 1998). Different plant genotypes exhibit traits that impact soil processes and feedbacks, but
84 because plant genotype and soil microbiome interact, soil microbial community responses to
85 climate change may also mediate plant responses to drought stress (Lau and Lennon, 2012;
86 Kaisermann et al., 2017). Therefore, understanding how stress-tolerant genotypes respond to
87 water stress also requires a better understanding of how microorganisms in their rhizospheres
88 respond to environmental variation.
89
90 By altering soil moisture content, soil aeration and the availability of soil nutrients, drought is
91 known to have direct negative effects on soil microbiota such as change in the microbial
92 community composition (Evans and Wallenstein, 2012; Preece and Peñuelas, 2016) and
93 decrease in microbial respiration rate and activities (Fierer et al., 2003; Moyano et al., 2013;
94 Meisner et al., 2017; Nguyen et al., 2018). Previous studies have confirmed that as water
95 content decrease soil environment become more favourable for fungi communities as
96 compare to bacteria (Hawkes et al., 2011; Manzoni et al., 2012). Moreover, microbial
97 communities are known to develop different physiological mechanisms in the face of drought
98 such as accumulation of solutes and the production of polysaccharides and spores (Schimel
99 et al., 2007; Allison and Martiny, 2008), providing resistance to drought stress. Because
100 different microbial taxa possess various tolerance degrees, long term exposure to water stress
101 may shift soil microbial community structure, selecting for resistant taxa capable of tolerating
102 the perturbation (Bouskill et al., 2013; Evans and Wallenstein, 2014). Therefore, the history of
103 water stress (legacy effect of the previous water exposure) can be important when attempting
104 to predict the degree to which plant and its associated microbiome respond to a subsequent

105 perturbation. For example, drought adapted microorganisms were shown to improve fruit and
106 flower numbers in *Brassica rapa* that were exposed to water stress (Lau and Lennon, 2012).
107 Yet, evidence from agro-ecosystems is scarce and the interaction between plant and soil
108 adaptations and the relative importance of the two processes on root-associated microbial
109 functions and abundance responses to a subsequent water stress are not well known (Lau
110 and Lennon, 2012; Kaisermann et al., 2017).
111
112 Here, we examined whether Canadian wheat genotypes, bred or not for drought resistance
113 growing in soils taken in directly adjacent wheat fields from the semi-arid region of
114 Saskatchewan that had been irrigated or not, would show different responses to decreased
115 soil water content in the abundance of microbes and processes rates in their rhizosphere. We
116 examined one general soil process, soil respiration (Griffiths et al., 2000) and one specialized
117 soil process, soil uptake of atmospheric H_2 (Constant et al., 2011) as well as bacterial and
118 fungal abundance in the rhizosphere of wheat plants exposed to four levels of SWC as follows:
119 high water content (50% soil water holding capacity, SWHC); moderate water content (30%
120 and 20% SWHC) and low water content (5-8% SWHC). We hypothesized that both the plant
121 genotype and soil water stress history will cause shift in the abundance of bacteria and fungi
122 in the rhizosphere which will have different functional consequences in the face of drought. As
123 such, the rhizosphere of wheat genotypes bred for drought tolerance growing in soils
124 previously exposed to drought are expected to show the highest functional resistance to
125 drought, leading to lower microbial functional change under low water availability. We further
126 hypothesized that responses will vary as a function of the guilds examined, with a more
127 pronounced response of a specialized soil process when compared to a general soil process.

128 ## 2. Materials and methods

129 ### 2.1. Soil sampling

130 Soil samples were collected from an experimental field located near Swift Current,
131 Saskatchewan (latitude: 50° 17′N; longitude: 107° 41′W) at the Swift Current Research and

132 Development Centre (SCRDC) of Agriculture and Agri-Food Canada. Soil managed under two
133 different irrigation regimes, one from an irrigated and another from a directly adjacent non-
134 irrigated experimental wheat field were collected (0-30 cm) in the spring of 2016, before
135 irrigation of experimental field start. These fields have been under a continuous 2-year rotation
136 (wheat-fallow) and the irrigated field is only irrigated during the wheat phase of the rotation
137 (every second year). Soil water content was similar for both soils (12.9% and 13.2% in non-
138 irrigated and irrigated soils, respectively, representing approximately 20% SWHC). No
139 significant difference was noted between the two soils for total nitrogen content and C/N ratio.
140 In the non-irrigated soil, average total nitrogen content and C/N ratio were 0.17% and 9.3,
141 respectively, while for the irrigated soil total nitrogen content and C/N ratio were 0.19% and
142 9.1, respectively. However, pH_{water} was significantly higher in the irrigated soil (6.1) compared
143 with the non-irrigated soil (5.3). After sampling, soils were sieved through a 2 mm sieve.
144

145 **2.2. Wheat genotypes**

146 Two Canadian wheat genotypes developed for Quebec climate: (1) Eastern spring wheat, AC
147 Nass (*Triticum aestivum*); (2) AC Walton (*Triticum aestivum*), and two genotypes developed
148 for Prairies climates: (3) Western spring wheat, AC Barrie (*Triticum aestivum*); (4) Western
149 Durum wheat, Strongfield (*Triticum turgidum subsp. durum)* were selected for this study.
150 Based on the Agriculture and Agri-Food Canada Research Centre, AC Nass and AC Walton
151 are characterized by high yield potential under favorable rain environments (Eastern Canada
152 climatic conditions), while AC Barrie and Strongfield exhibit higher yield under lower
153 precipitation regimes (Canadian Prairie climatic conditions).
154

155 **2.3. Experimental design**

156 The experiment consists of the following treatments: 4 wheat genotypes × 2 different soil types
157 × 4 levels of SWC: high SWC (50% of soil water holding capacity (SWHC)); moderate SWC
158 (30% and 20% SWHC) and low SWC (5-8% SWHC). Five replicate pots (14.5 cm high × 19
159 cm diameter) were filled with approximately 1 kg of soil, and eight seeds of each wheat

160 genotypes were sown (32 treatments × 5 replicates = 160 pots). Pots were placed in the plant
161 growth room based on a randomised complete block design under controlled conditions of
162 16:8-h light: dark cycle, 22-24°C and 800 μmol^{-2}s^{-1} photon flux density. All pots were kept at
163 50% SWHC for 4 weeks and were watered uniformly every day (between 8-10 AM) until the
164 start of the experiment. After one month, soil moisture treatments were applied by adjusting
165 SWC to 5-8% SWHC, 20% SWHC, 30% SWHC while controls were kept at 50% SWHC. Pots
166 were weighed every day (between 8-10 AM) to monitor the soil water content at the target
167 level by adding the correct amount of sterile deionized water. After four weeks of growth under
168 different moisture regimes rhizosphere, root and shoot samples were collected. The roots
169 were shaken to remove the loose soil and the remaining attached soil, considered to be the
170 rhizosphere soil, was collected by using sterile brushes. Plant biomass was divided into root
171 and shoot portions, and then weighed to obtain fresh weight and dried at 75 °C for 48 h to
172 obtained dry weight. The following microbial parameters were measured in the rhizosphere
173 samples: CO_2 production and H_2 consumption rates, the total abundance of bacteria (qPCR
174 targeting the 16S rRNA gene) and fungi (qPCR targeting the ITS region). A subsample of
175 rhizosphere soil from each treatment was oven dried at 75 °C for 48 h to determine the
176 moisture content.
177

178 **2.4. DNA extraction and qPCR assays**

179 Genomic DNA was extracted using a phenol-chloroform extraction method (Dellaporta et al,
180 1983). Soil samples (0.5 g fresh weight) were transferred to 2 ml micro-centrifuge tubes
181 containing approximately 700 mg of glass beads (a mix of 0.1 mm and 0.5mm zirconia-silica
182 beads). About 1000 μL of extraction buffer (10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA (pH 8.0)
183 and 20 μl 20% SDS (wt/vol)) was added to each tube. After bead beating (FastPrep24, MP
184 Biomedicals; 45 s, 6.5ms^{-1}) and spinning down the debris (15,000 × g, 15 min, 4°C), the
185 supernatant was transferred to new tube and 500 μl of 25:24:1 phenol:chloroform:isoamyl
186 alcohol was added, mixed and centrifuged (15,000 × g, 10 min, 4 °C). The aqueous phase
187 was then mixed with 500 μl of 24:1 chloroform:isoamyl alcohol, mixed and centrifuged (15,000

188 × g, 2 min, 4°C). The aqueous phase was collected and the DNA was stabilized with 50 µL of
189 ammonium acetate (3 M), followed by precipitation by adding 1ml ice-cold 70% ethanol and
190 further stored at -80°C for 2 h. After centrifugation at 15,000 × g for 15 min, the supernatant
191 was discarded, DNA pellets were washed with 200 µL ice-cold 70% ethanol, and tubes were
192 centrifuged at 15,000 × g for 2 min. The ethanol was then discarded, the pellets air-dried at
193 room temperature for 30 min and resuspended in 100 µl DNA-free water and stored at -20°C.
194 DNA quantity and quality was measured by UV absorbance using a NanoDrop ND-1000
195 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) at 230, 260 and 280 nm
196 (respectively A230, A260 and A280).
197
198 Real-time quantitative PCR was performed on the DNA extracted from the rhizospheric soil to
199 estimate the total abundance of bacteria (16S rRNA genes) and fungi (ITS regions). For the
200 quantification of the 16S rRNA gene, standard curves were made by diluting serially a nearly
201 full-length amplicon of *Escherichia coli* 25922 (American Type Culture Collection, Manassas,
202 VA, USA) produced using primers PA-27F-YM and PH-R (Bruce et al., 1992). For the
203 estimation of fungal abundance, the standard curves were created by serial dilutions of the
204 ITS region amplified from the yeast *Pichia scolyti* by the primers NSA3 and NLC2 (Martin &
205 Rygiewicz, 2005). For the qPCR reaction, the primers Eub338-F and Eub518-R (Fierer et al.,
206 2005) were used for bacteria, whereas ITS1-F and 582AR were used for fungi (Martin &
207 Rygiewicz, 2005). The qPCR reactions were performed on a RotorGene 6000 machine
208 (Corbett Research, Mortlake, NSW, Australia) using the SsoAdvanced^{TM} Universal SYBR
209 Green kit (Biorad, Hercules, CA, USA). For both genes, each reaction mix contained the
210 following: 4.2 µl sterilized water, 10 µl SYBR green mastermix, 0.4 µl of each primer (0,4
211 pmoles/µl) and 5 µl template DNA for a total reaction volume of 15 µl. Each qPCR run had at
212 least two no template controls. The PCR conditions consists of an initial denaturation step at
213 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C
214 for 30 s and elongation at 72°C for 30 s. Fluorescence was measured at the end of each cycle
215 at the elongation step. A melt curve analysis was done to verify the specificity of the amplicons.

2.5. CO_2-flux measurements

Microcosms containing rhizosphere samples (5 g dry wt soil) were closed tightly with rubber septum caps. To monitor CO_2 net respiration in the microcosms, headspace samples (10 cm^3) were collected with syringe and injected through the injection port of a gas chromatograph (Agilent 7890 A, Agilent Technologies, Santa Clara, CA, USA). CO_2 effluxes were regularly measured every 24 h for 3 d at 24°C. Soil CO_2 efflux was then calculated from the slope of the change in CO_2 concentration over time at time points 10, 1440 and 2880 minutes, using linear regression. The gas chromatograph was calibrated before measurement using certified CO_2 standard gas mixture (620 ± 10 ppmv CO_2, GSTWelco, Pennsylvania, USA) and standard deviations of repeated analyses were <5%. In this article, the terms CO_2 flux and CO_2 production are interchangeably used, referring to net exchange rate measured in the gas chromatographic assay.

2.6. H_2 soil uptake activity

Soil gas exchanges were measured as described by Khdhiri et al. (2015). Microcosm containing rhizosphere samples (5 g dry wt soil) were tightly closed with rubber septum caps. 3 cm^3 of H_2 gas mixture containing 525 ± 10 ppmv H_2 (GST-Welco, PA, USA) was injected to headspace of the microcosms in order to obtain the range of initial concentration of 2-3 ppmv H_2. To monitor decrease of H_2 mixing ratio in the microcosms, the headspace was sampled by removing 10 cm^3 of headspace gas into syringe and then injected through the injection port of a gas chromatograph equipped with a reduction gas detector (ta3000R, Ametek Process Instruments®, Delaware, USA). H_2 oxidation rate was calculated by integrating the decline of headspace H_2 ratio by using three H_2 concentration points for data integration. The analyser was calibrated before measurement using certified H_2 standard gas mixture (2.13 ppmv ± 5%, GSTWelco, Pennsylvania, USA) and standard deviations of repeated analyses were <5%. No significant change in H_2 concentration was detected for blank samples (empty microcosms that did not receive sample).

244

245 **2.7. Statistical analyses**

246 The comparison of the regression lines for the four plant genotypes was used to check for the
247 possible effect of wheat genotype on the impact of SWC on microbial (gas flux analysis and
248 the relative abundance of total bacteria and fungi) and plants (shoot and root biomass)
249 parameters. In order to differentiate the response pattern of each plant genotype per soil type
250 over the different moisture treatments, in the regression analysis the four plant genotypes
251 were analysed for irrigated and non-irrigated soils separately. In addition, the comparison of
252 regression lines of two soils (irrigated and non-irrigated soils) was performed to verify the
253 possible effect of irrigation legacy on the impact of SWC on shoot and root biomass, gas flux
254 analysis and the relative abundance of total bacteria and fungi. CO_2 production data were
255 square root transformed in order to satisfy the assumptions of normality and multivariate
256 normality. Statistical analyses were performed using Statgraphics Centurion XV software
257 (StatPoint).

258

259 **3. Results**

260 **3.1. Plant biomass**

261 **3.1.1. Shoot biomass**

262 In general, shoot biomass decreased significantly with decreasing SWC for plants growing in
263 both irrigated ($p < 0.001$ for SWC; Fig. 1A) and non-irrigated soils ($p < 0.001$ for SWC; Fig.
264 1B). However, the magnitude of the effect of SWC on shoot biomass reduction was different
265 depending on soil type and plant genotype. The comparison of regression lines revealed that
266 although shoot biomass of all genotypes growing in irrigated soils did not differ ($p = 0.917$ for
267 the intercepts), SWC affected the shoot biomass of wheat genotypes to a different extent ($p <$
268 0.001 for the slopes; Fig. 1A). For example, under low water content the shoot biomass of AC
269 Nass decreased more (82% reduction of the biomass relative to the well-watered controls)
270 than the shoot biomass of AC Barrie (50% reduction) (Fig. 1A). However, in case of plants

271 growing in non-irrigated soils, SWC affected the shoot biomass of wheat genotypes to a similar
272 extent ($p = 0.083$ for the slopes), and no significant difference was noted between genotype
273 ($p = 0.446$ for the intercepts; Fig. 1B). Overall, shoot biomass reduction was stronger in soil
274 with history of drought stress (non-irrigated soils) compared with those with no recent history
275 of water stress (irrigated soils) ($p = 0.009$ for the intercepts; Fig. 1C).
276
277 **3.1.2. Root biomass**
278 Similar to the shoot biomass, we found significant effect of SWC on root biomass for plants
279 grown in both irrigated ($p < 0.001$ for SWC; Fig. 1D) and non-irrigated soils ($p < 0.001$ for
280 SWC; Fig. 1E). For the effect of SWC on root biomass of plants growing in irrigated soils
281 significant difference was found between genotype ($p = 0.003$ for the regression intercepts)
282 but in general, SWC affected root biomass of all genotypes to a similar extent ($p = 0.391$ for
283 the slopes; Fig. 1D). For instance, the root biomass of AC Barrie and Strongfield decreased
284 significantly (48% and 30% reduction of the biomass in the 30% SWHC treatment relative to
285 the well-watered controls, respectively), but root biomass of AC Nass and AC Walton
286 increased (86% and 66% increase, respectively) (Fig. 1D). In case of plants growing in non-
287 irrigated soils, SWC affected root biomass of wheat genotypes in a different way ($p = 0.055$
288 for the slopes), and significant difference was observed between genotype response ($p =$
289 0.039 for the intercepts). For example, in comparison with well-watered control, at 30% SWHC
290 root biomass increased in all genotypes (except for Strongfield), and this trend was more
291 pronounced for AC Walton (71% increase of the biomass in the 30% SWHC treatment relative
292 to the well-watered controls) as compared to AC Nass (22% increase) (Fig. 1E). These results
293 indicate different response pattern of genotypes growing in soil with different water stress
294 exposure history. In contrast to the shoot biomass, plants grown in non-irrigated soils had
295 higher root biomass under 50% SWHC and 30% SWHC in comparison with plants grown in
296 irrigated soils ($p < 0.001$ for the intercepts; Fig. 1F). Moreover, under low water content, root
297 biomass of all plants growing in irrigated and non-irrigated soils decreased due to lower water
298 content as compared with well-watered control (Fig. 1F).

3.2. qPCR quantification of the bacterial 16S rRNA gene

Significant effect of SWC on the 16S rRNA gene copy number in the rhizosphere of plants grown in irrigated soils was observed ($p < 0.001$ for SWC; Fig. 2A), however SWC effect was not significant for non-irrigated soils ($p = 0.121$ for SWC; Fig. 2B). These results indicate a significant legacy effect of previous water stress exposure and some degree of resistance in non-irrigated soils in face of subsequent water stress. In addition, regression analyses showed a significant difference between the 16S rRNA gene copy number in the rhizosphere of plants growing in irrigated soils ($p < 0.001$ for the intercepts) and SWC affected the 16S rRNA gene copy number of wheat genotypes to a different extent ($p = 0.003$ for the slopes; Fig. 2A). We found that with decreasing SWC the copy number of 16S rRNA genes changed less in the rhizosphere of AC Nass and Strongfield, whereas it decreased significantly in the rhizosphere of AC Barrie and AC Walton (decrease from 6.7×10^7 and 6×10^7 copies (g^{-1} soil-dw) in the 50% SWHC treatment to 4.8×10^7 and 4.5×10^7 copies (g^{-1} soil-dw) in the 5-8% SWHC treatment in the rhizosphere of AC Barrie and AC Walton, respectively) (Fig. 2A). In general, long-term irrigation enhanced the 16S rRNA gene copy number: with the exception of Strongfield, the abundance of bacterial 16S rRNA gene was higher in irrigated soils than in non-irrigated soils ($p < 0.001$ for the intercepts; Fig. 2C), and the effect was more pronounced in the rhizosphere of AC Walton (Fig. 2A, B)

3.3. qPCR quantification of the fungal ITS region

Interestingly, no significant effect of SWC on the abundance of fungal ITS regions was noted for both irrigated ($p = 0.475$ for SWC; Fig. 2D) and non-irrigated soils ($p = 0.143$ for SWC; Fig. 2E), indicating the resistance of fungi to water stress. Significant difference between the abundance of fungal ITS regions in the rhizosphere of plants growing in irrigated soils was detected ($p < 0.001$ for the intercepts) but SWC affected the abundance of fungal ITS regions in the rhizosphere of wheat genotype to the same extent ($p = 0.653$ for the slopes; Fig. 2D). In general, the abundance of fungal ITS regions were higher in the rhizosphere of AC Nass

Commented [ÉY1]: Maybe have 3.2: qPCR quantification, 3.2.1 Bacterial 16S rRNA gene, … and then 3.3. Gas flux measurement, 3.3.1 CO2, etc. Would match better the paragraph structure for the plant biomass.

327 and Strongfield in comparison with other genotypes (Fig. 2D). The abundance of fungal ITS
328 regions in the rhizosphere of plants growing in non-irrigated soils varied significantly between
329 wheat genotypes ($p < 0.001$ for the intercepts), with the distinction between the abundance of
330 fungal ITS regions in the rhizosphere of plant genotype in response to various SWC ($p < 0.001$
331 for the slopes; Fig. 2E). For instance, in the rhizosphere of Strongfield grown in soil that had
332 been subjected to previous drought, the abundance of fungal ITS increased with decreasing
333 SWC (increases from 1.4×10^7 copies (g^{-1} soil-dw) in the 50% SWHC treatment to 5×10^7
334 copies (g^{-1} soil-dw) in the 5-8% SWHC treatment) (Fig. 2E). However, the opposite trend was
335 observed for AC Walton where the abundance of fungal ITS decreased with decreasing water
336 content (Fig. 2E). Moreover, in contrast to 16S rRNA gene copy numbers, the rhizosphere of
337 plants growing in non-irrigated soils tended to have higher copy numbers of fungal ITS regions
338 compared with those in irrigated soils ($p = 0.017$ for the intercepts; Fig. 2F), except for AC
339 Nass where irrigated soils showed higher copy numbers of fungal ITS regions compared with
340 those in non-irrigated soils (Fig. 2D, E)
341

342 **3.4. CO_2 production**

343 We found a significant effect of SWC on CO_2 production in the rhizosphere of plants growing
344 in irrigated ($p < 0.001$ for SWC; Fig. 3A) and non-irrigated soils ($p < 0.001$ for SWC; Fig. 3B).
345 However, no significant difference between CO_2 production in the rhizosphere of plant
346 genotype growing in irrigated soils was detected ($p = 0.776$ for the intercepts) and the slope
347 of regression lines were not significantly different between wheat genotypes ($p = 0.946$ for the
348 slopes), meaning that SWC affected CO_2 production in the rhizosphere of wheat genotype to
349 the same extent (Fig. 3A). However, CO_2 production in the rhizosphere of plants growing in
350 non-irrigated soils varied significantly between wheat genotypes ($p = 0.047$ for the intercepts),
351 and no distinction observed between CO_2 production in the rhizosphere of plant genotype in
352 response to various SWC ($p = 0.911$ for the slopes; Fig. 3B). For instance, at 50% and 30%
353 SWHC, CO_2 production in the rhizosphere of Strongfield and AC Nass growing in non-irrigated
354 soils was higher than in the rhizosphere of other genotypes, whereas at 20% SWHC the

355 rhizosphere soil of AC Walton produced significantly less CO_2 than other genotypes (Fig. 3B).
356 However, under low water content, the rhizosphere of all plants produced similar amounts of
357 CO_2, irrespective of soil water stress history (Fig. 3A, B). No significant difference was
358 observed in CO_2 production in the rhizosphere of plants growing in irrigated soil as compared
359 with the rhizosphere of plants growing in non-irrigated soils ($p = 0.869$ for the intercepts) and
360 both soils responded in a similar way under different SWC ($p = 0.495$ for the slopes; Fig. 3C).
361 These results suggest no legacy effect on general soil process (CO_2 emissions).
362

363 **3.5. H_2 uptake activity**

364 Significant effect of SWC on H_2 oxidation rates in the rhizosphere of plants grown in irrigated
365 soils was observed ($p = 0.005$ for SWC; Fig. 3D), however SWC effect was not significant for
366 non-irrigated soils ($p = 0.173$ for SWC; Fig. 3E). The comparison of the regression lines
367 revealed different pattern in H_2 oxidation rates in the rhizosphere of wheat genotype grown in
368 irrigated soils ($p = 0.012$ for the intercepts) and the slopes of the regression lines were
369 significantly different between H_2 oxidation rates in the rhizosphere of plant genotype in
370 response to SWC ($p < 0.001$ for the slopes; Fig. 3D). For AC Barrie with decreasing SWC, H_2
371 uptake rates increased in the rhizosphere of plants growing in the irrigated soil (386 pmol $^{h-1}$
372 $g_{(dw)}^{-1}$ for 50% SWHC and 662 pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 5-8% SWHC), and to a lesser extent AC
373 Walton (455 pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 50% SWHC and 526 pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 5-8% SWHC), whereas
374 oxidation rates remained to some extent stable for other plants genotypes (Fig. 3D). As for
375 non-irrigated soil, with decreasing SWC, H_2 uptake rates increased in the rhizosphere of AC
376 Walton (494 and pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 50% SWHC and 580 and pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 5-8% SWHC).
377 However, the opposite pattern was observed for AC Nass, where with decreasing SWC, H_2
378 uptake rates decreased (353 and pmol $^{h-1}$ $g_{(dw)}^{-1}$ for 50% SWHC and 210 and pmol $^{h-1}$ $g_{(dw)}^{-1}$ for
379 5-8% SWHC) (Fig. 3E). These results indicate significant difference between H_2 uptake activity
380 in the rhizosphere of plants grown in non-irrigated soils ($p < 0.001$ for the intercepts) and
381 further, distinction between H_2 oxidation rates in the rhizosphere of plant genotype in face of
382 different SWC ($p = 0.001$ for the slopes; Fig. 3E). In contrast to CO_2 production, we observed

383 higher H_2 oxidation rates under low water content in the rhizosphere of plants growing in
384 irrigated soil as compared with the rhizosphere of plants growing in non-irrigated soils (Fig.
385 3C).
386

387 **4. Discussion**

388 This study aimed at unravelling how different wheat genotypes, bred or not for increased
389 resistance to drought and different semi-arid soils, with or without a history of water stress
390 independently or interactively influence the response of wheat and its associated rhizosphere
391 microbiome to water stress. Our initial hypothesis that wheat genotypes bred for drought
392 tolerance growing in soils previously exposed to drought would show the highest resistance
393 to water stress and lower microbial functional change under low water availability was not
394 verified. Our results indicate that wheat genotypes and soil water stress histories affected
395 microbial abundance and functional processes in the rhizosphere, while also modifying wheat
396 biomass. These results are important as they suggest that wheat adaptation to stress is partly
397 reliant on soil microbes, and that microbiome management is likely an efficient complement to
398 plant breeding when aiming at improving wheat resistance to stress.
399

400 Soil water limitation is known to affect plant biomass and alter biomass reallocation, which all
401 depend on plant species and duration and intensity of water stress (McDowell, 2011;
402 Sanaullah et al., 2011; Xue et al., 2017). In general, through allocation of C pools to the roots,
403 water stress resulted in limited shoot growth and larger root systems (Liu and Li, 2005) in order
404 to sustain water and nutrient uptake (Kaisermann et al., 2017). Higher root biomass also
405 results in increases in carbon input to the soil (Preece and Peñuelas, 2016). However, an
406 increase in root growth becomes less likely under low water content (5-8% SWHC) when
407 threshold level of water limitation has been reached. This is consistent with a study showing
408 that fine root length increased under moderate soil water content (at a soil water potential of
409 –0.4 MPa), but decreased at low soil water content (at a soil water potential of –1.0 MPa) in

410 beech saplings (*Fagus sylvatica*) (Zang et al. 2014). Here, we showed that under moderate
411 and low SWC the wheat shoot biomass was lower when growing in soils that had been
412 subjected to long-term water stress (non-irrigated soil). In contrast, under moderate water
413 contents wheat grown in soils with a history of water stress (non-irrigated soil) had a
414 significantly higher root biomass compared with those grown in soil with no history of water
415 stress (irrigated soils). This suggests that the commonly observed increased allocation of
416 resources to root growth under moderate water stress depends, at least partly, on the previous
417 exposure to water stress of the soil microbiome. Indeed, soil carbon and nitrogen contents
418 were statistically identical for both soils, but we observed some interesting differences in pH
419 and bacterial abundance. The irrigation legacy effect thus involved a combination of biotic and
420 abiotic factors, including alteration in soil ionic exchange capacity and nutrient availability for
421 plant and microbes.
422
423 Adaptation of microbes to drought conditions in the long-term field experiment was depicted
424 by bacterial abundance profiles, as we observed some degree of resistance in soil previously
425 exposed to water stress (non-irrigated soils) with smaller decreases with decreasing soil water
426 content when compared to soils not previously exposed to water stress (irrigated soils).
427 Resistance to water stress might occur because of the survival of soil microorganisms capable
428 of tolerating stress in soils that had previously been subjected to this stress (Wallenstein &
429 Hall, 2012; Bouskill et al., 2013; Hawkes & Keitt, 2015). Our results show that this microbial
430 resistance persists in time and can influence root-to-shoot ratio of a subsequent generation of
431 wheat, making it better adapted and ready to face water stress (Kaisermann et al., 2017). This
432 conclusion is consistent with a study along long-term rainfall manipulation by Evans and
433 Wallenstein (2012), which showed that long-term drought exposure improved the response of
434 microbial communities to an additional water stress in comparison with communities that were
435 not previously exposed to water stress (Evans and Wallenstein, 2014). In line with this, Lau
436 and Lennon, (2012) reported that drought adapted microorganisms were able to improve fruit
437 and flower numbers in *Brassica rapa* that were exposed to water stress. Therefore, wheat

438 response to water stress is not solely controlled by plant genotype, but is also tributary of the
439 associated soil microbes, opening up potential new avenues for improving wheat resistance
440 to environmental stresses.
441
442 In addition to influencing bacterial abundance response to water stress, soil previous water
443 stress exposure influenced differently a general and a specialized functional process. We
444 observed that the rhizosphere of wheat growing in both soils released the same amount of
445 CO_2 under low soil water content. The reduced CO_2 production with decreasing soil water
446 content in our study is in line with the findings of others (Fierer et al., 2003; Manzoni et al.,
447 2012; Nguyen et al., 2018). However, at low water content we found significantly higher H_2
448 oxidation rates in the rhizosphere of plants growing in the irrigated soil as compared to the
449 non-irrigated soils. These results indicate that previous exposure to water stress not only
450 affects total bacterial abundance to an additional water stress, but also the microbial
451 communities responsible for a specialized function, resulting in different soil functional process
452 rates under water stress, which might have far reaching consequences considering the broad
453 taxonomic and functional diversity of high affinity H_2-oxidizing bacteria (Constant et al., 2011;
454 Greening et al., 2016). Current findings suggest that there is high functional redundancy
455 among soil microbiota (Wolters, 2001; Epelde et al., 2010), but the fact that numerous
456 microbes can carry out a function does not necessarily mean that the process rates under
457 stressful conditions will be identical, as it has been shown here.
458
459 On top of the differences observed between wheat plants and its associated microbiome
460 growing in soils with different water stress history, wheat genotypes also influence some of
461 the response patterns to water stress. Similarly, it has been shown that identical water stress
462 treatments have inconsistent effects on different plant species and even at the sub-species
463 level (Sanaullah et al., 2011; Preece and Peñuelas, 2017). For example, shoot biomass of
464 *Festuca arundinacea* and *Medicago sativa* decreased significantly under water stress
465 conditions (30% of the field capacity) compared to the same plants grown under optimum

466 water level (70% of the field capacity), whereas shoot biomass was unaffected for *Lolium*
467 *perenne* (Sanaullah et al., 2011). Part of the wheat genotype effect observed could be linked
468 to rhizodeposition/root exudation. Indeed, it has been shown that the C compounds released
469 by plant roots are highly dependent on plants species and even vary between plant genotypes
470 and a large part of these compounds (64-86%) are rapidly respired by the rhizosphere
471 microbiome (Hütsch et al., 2002). Accordingly, although CO_2 production in the rhizosphere of
472 all genotypes decreased with decreasing SWC, the extent to which CO_2 production decreased
473 between treatments in non-irrigated soils depended on genotypes. Plant biomass also
474 influences the amount and composition of rhizodeposition (Dijkstra and Cheng, 2007; Zhu and
475 Cheng, 2013), resulting in shifts in the rates of CO_2 production by root-associated microbial
476 communities.
477
478 In accordance to the decrease in CO_2 emissions with decreasing soil water content, the gene
479 abundances of bacterial 16S rRNA genes (in the rhizosphere of AC Walton and AC Barrie)
480 decreased with decreasing soil water content in soils with no history of water stress (irrigated
481 soils). These findings are consistent with those from previous studies showing decreases in
482 the abundance of bacteria with increasing soil dryness (Maestre et al., 2015). Shift in the
483 abundance of bacterial16S rRNA genes may be driven by a direct negative effect of soil water
484 content, as bacterial activity needs a constant supply of water (Schimel et al., 2007; Ngumbi
485 and Kloepper, 2016). Another possible explanation for decline in the abundance of bacteria
486 could be due to competitive interactions between fungi and bacteria in the face of drought. In
487 general, the abundance of fungi did not change significantly under different water content in
488 both irrigated and non-irrigated soils, likely due to strong cell walls (Schimel et al., 2007) and
489 capability to transport water through the hyphal network (Khalvati et al. 2005). Fungal species
490 are known to respond to different moisture conditions (Hawkes et al., 2011) but in comparison
491 to bacteria, fungi are often able to better cope with water limitation (Manzoni et al., 2012;
492 Fuchslueger et al., 2014) and may outcompete bacteria, reducing their abundance as a
493 consequence of competition for resource and other negative interactions. For example, a

study which was conducted along rainfall manipulations field in a northern California grassland (between 2005 and 2008) showed that whereas fungal communities remained stable (June 2006), a significant change was noted in bacterial communities across altered rainfall treatments (April and July 2006), likely due to interactions between bacteria and fungi (Hawkes et al., 2011). It is thus important to consider, and more importantly differentiate, the relative importance of the direct and indirect effects of changing environments on soil-plant-microbe interactions.

Commented [ÉY2]: Not clear what you mean here. The fungi are unaffected and bacteria are affected, and this is due to interactions? Could it be that bacteria are simply more sensitive?

It should be noted however that our study was conducted using one soil for each of the two irrigation history. Further study integrating more soils with a wider range of irrigation histories will help to confirm the results presented here and clarify if drought-tolerant microbes can help plant to withstand subsequent water stress.

Commented [ÉY3]: Can you move this somewhere else. It makes it weaker to finish off on a caveat statement. Maybe move it where you talk about the soil legacy effect?

5. Conclusions

In conclusion, our results demonstrate intimate relationships between wheat genotypes and soil water stress history in shaping the responses of root-associated microorganisms and wheat biomass to decreasing soil water content. It is becoming more evident that plant breeding is not the only key to mitigate crop yield losses due to stress, but that microorganisms might also play a major role to improve wheat yields under environmental stresses. Our study is aligned with this emerging paradigm shift, as our results showed that wheat genotypes and soil water stress histories affected microbial abundance and functional processes in the rhizosphere, while also modifying wheat biomass. Therefore, understanding how wheat genotypes respond to soil drying, also requires understanding how microorganisms in their rhizospheres respond to environmental variation, and what was their previous exposure to environmental stresses. This study has implications for improving our understanding of the drivers of soil processes in the rhizosphere of wheat, knowledge that will be essential to mitigate negative effects of climate changes on crop productivity.

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674

675

676

677 **Figure captions:**

678 **Fig. 1.** Effect of soil water content on shoot (A, B, C) and root (D, E, F) biomass of four wheat
679 genotypes grown in semi-arid soils with different irrigation histories. Soil water content (SWC)
680 refers to four level of soil water holding capacity: high SWC (50% SWHC); moderate SWC
681 (30% and 20% SWHC) and low SWC (5-8% SWHC). Genotype refers to AC Nass (*Triticum*
682 *aestivum*), AC Walton (*Triticum aestivum*), AC Barrie (*Triticum aestivum*), Strongfield (*Triticum*
683 *turgidum* subsp. durum). Soil type refers to soils with either an history of water stress (NI: non-
684 irrigated soils from Saskatchewan) or with no recent history of water stress (IR: irrigated soils
685 from Saskatchewan).

686

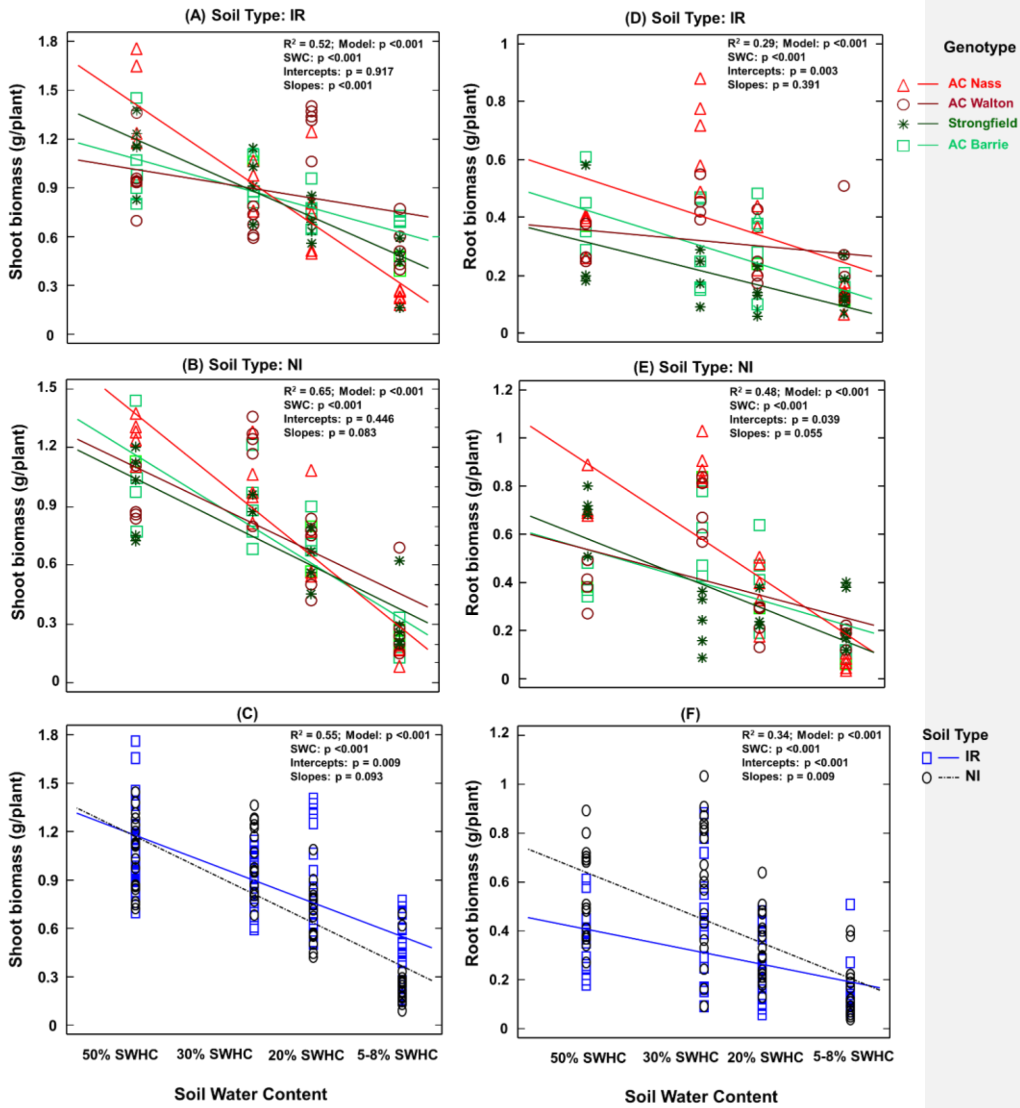
687 **Fig. 2.** Effect of soil water content on the abundance of bacterial 16S rRNA gene (A, B, C) and
688 the abundance of fungi (D, E, F) in the rhizosphere of four wheat genotypes grown in semi-
689 arid soils with different irrigation histories. See Fig.1 for more information.

690

691 **Fig. 3.** Effect of soil water content on a general (A, B, C: CO_2 production) and a specialized
692 (D, E, F: soil uptake of atmospheric H_2) functional process in the rhizosphere of four wheat
693 genotypes grown in semi-arid soils with different irrigation histories. See Fig.1 for more
694 information.

695

696



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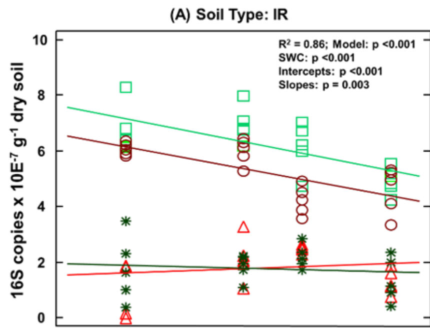
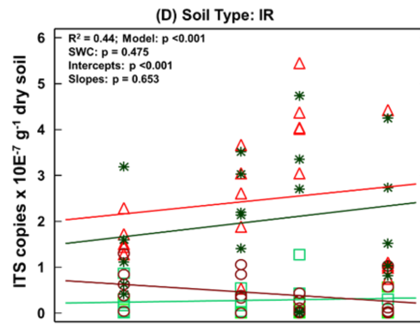
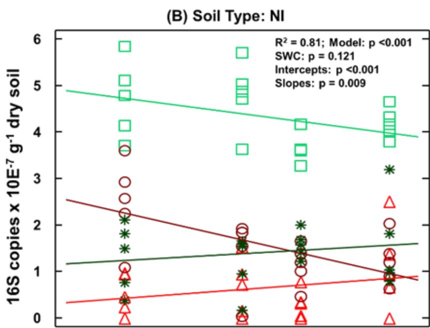
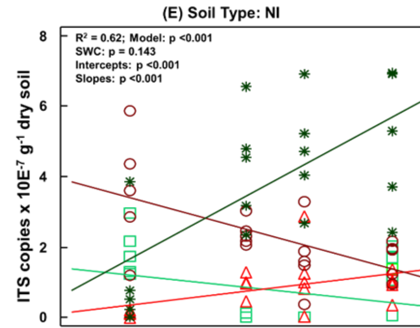
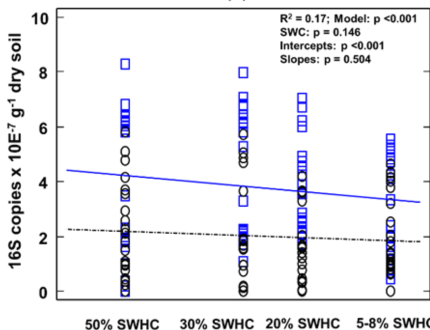


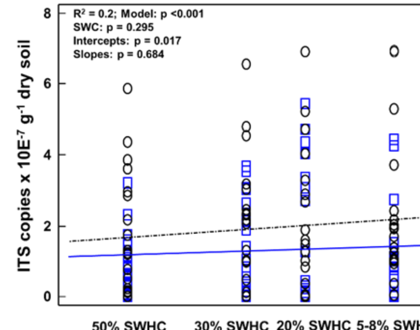


Genotype
AC Nass
AC Walton
Strongfield
AC Barrie









Soil Type
IR
NI

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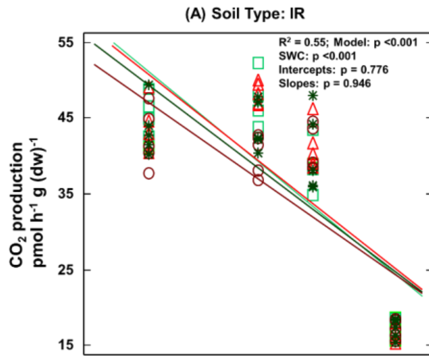

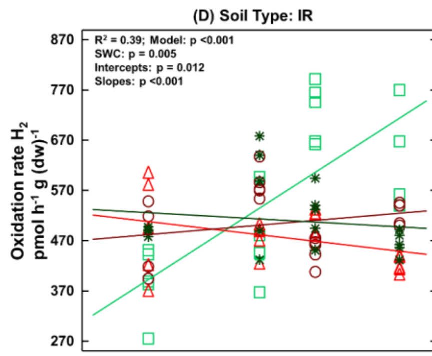

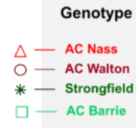

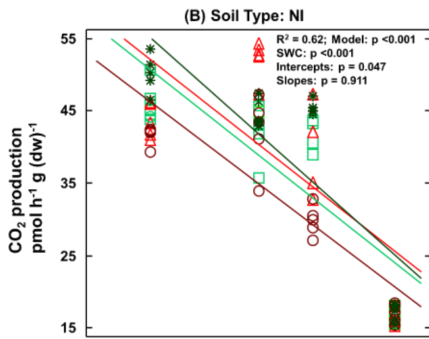

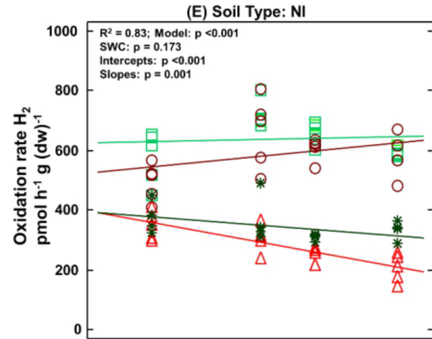

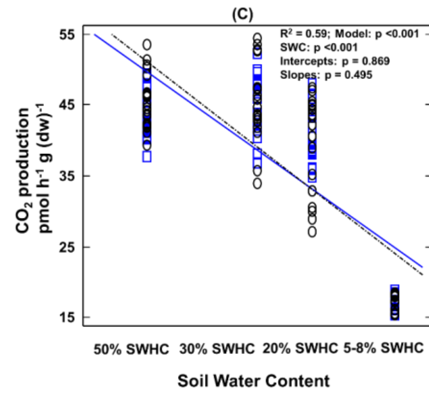

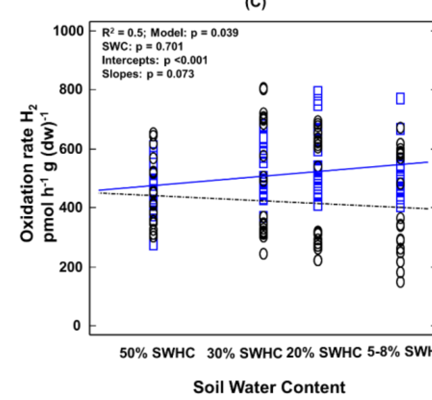

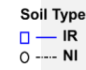

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