

## Aberystwyth University

### *Reduced soil respiration beneath invasive *Rhododendron ponticum* persists after cutting and is related to substrate quality rather than microbial community*

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1 **Reduced soil respiration beneath invasive *Rhododendron ponticum* persists after cutting and is**  
2 **related to substrate quality rather than microbial community.**

3

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21

22 **Abstract**

23 Invasive plants depositing recalcitrant, polyphenol-rich leaf litter may alter decomposition dynamics,  
24 leading to an accumulation of soil organic matter. Removing invasives is critical in restoring native  
25 habitats, but our understanding of its impacts upon soil processes remains limited. Here, we test the  
26 hypothesis that clearing of *Rhododendron ponticum* leads to increased soil respiration, at a site  
27 within Snowdonia National Park, Wales, UK. Soil samples were collected, and soil respiration was  
28 monitored over 32 weeks on plots cleared of *R. ponticum*, plots infested with *R. ponticum* which  
29 were left uncleared, and uninvaded plots of native vegetation. Soil respiration was significantly  
30 higher in native vegetation plots, relative to uncleared plots. Clearing *R. ponticum* led to a short-  
31 term (< four weeks) increase in soil respiration relative to uncleared plots and was related to  
32 elevated soil temperature post-clearance. However, this respiration response was transient, with no  
33 significant difference between cleared and uncleared plots over the whole growing season (32  
34 weeks). Declining soil respiration responses to soil warming have been attributed to altered soil  
35 microbial communities and substrate limitation. Analysis of microbial phospholipid fatty acids  
36 (PLFAs) detected no differences among cleared, native and uncleared plots post-clearance. However,  
37 Fourier-transform mid-infrared spectroscopy detected a decline in organic matter aromaticity over  
38 the growing season in the native and uncleared plots, whilst there was no change in the cleared  
39 plots. The findings show that despite a pulse in soil respiration during the initial four weeks post-  
40 clearance, *R. ponticum* litter and associated soil organic matter in cleared plots continued to  
41 decompose at a similar rate to uncleared plots over the whole growing season. This was likely a  
42 result of substrate limitation and altered soil organic matter composition following *R. ponticum*  
43 clearing, with labile carbon becoming depleted and an enrichment of more recalcitrant aromatic  
44 structures.

45 **Keywords:** "Invasive"; "soil"; "respiration"; "decomposition"; "PLFAs"; "FTIR"; "soil organic matter".

46

47 **1. Introduction**

48 Invasive plants can alter soil processes such as soil organic matter (SOM) decomposition by  
49 depositing high quantities of chemically distinct litter (Suseela et al., 2016; Tamura et al., 2017). The  
50 decomposition of leaf litter is determined by three main factors: litter chemical composition, soil  
51 physicochemical properties and the microbial community (Jewell et al., 2015). Plant invasions have  
52 the potential to alter all three of these factors, with litter chemistry particularly important (Pattison  
53 et al., 2016; Suseela et al., 2016; Tamura et al., 2017). Plants adapted for high-nutrient environments  
54 produce labile litter which rapidly decomposes and leads to lower soil carbon content post-invasion  
55 (Liao et al., 2008; Tamura and Tharayil, 2014). Conversely, plants adapted for low-nutrient  
56 environments produce low-quality litter, which is rich in phenolic compounds (Aerts, 1999; DeLuca  
57 et al., 2013; Hobbie, 1992). This recalcitrant litter decomposes slowly, leading to altered soil  
58 microbial communities, shifts in SOM composition and increased carbon sequestration (Ehrenfeld et  
59 al., 2001; Suseela et al., 2016; Tamura and Tharayil, 2014).

60 Altering soil processes may be important for certain invasive shrubs, such as the UK invasive plant  
61 *Rhododendron ponticum* L., given that ericaceous species leaf litter strongly influences  
62 decomposition dynamics (Aerts, 1997; DeLuca et al., 2013). It is known that related *Rhododendron*  
63 spp. produce acidic, polyphenol rich litter which decomposes slowly (Monk et al., 2014; Wurzburger  
64 and Hendrick, 2007). Furthermore, tannins leaching from the litter of *Rhododendron* spp. can inhibit  
65 microbial activity, form decay-resistant complexes with proteins and inactivate enzymes in the  
66 underlying soil (Hättenschwiler and Vitousek, 2000; Horner et al., 1988; Wurzburger and Hendrick,  
67 2009). The slow decomposition of *Rhododendron* spp. litter results in the formation of a thick layer  
68 of undecomposed litter on the soil surface (Plocher and Carvell, 1987) and an accumulation of SOM  
69 (Wurzburger and Hendrick, 2007).

70 Whilst many studies have observed altered decomposition dynamics beneath invasive plants, few  
71 have looked at how clearing invaded sites affects soil processes (Frank et al., 2018; Osburn et al.,

72 2018). Recent years have seen a considerable effort to control the spread of invasives such as *R.*  
73 *ponticum* and restore native habitats in the UK (Jackson, 2008; Snowdonia Rhododendron  
74 Partnership, 2015; Tyler et al., 2006). The most common method of control for large patches of  
75 mature *R. ponticum* thickets is to cut and either burn or chip the stems, whilst periodically revisiting  
76 sites to apply herbicide on *R. ponticum* regrowth (Edwards, 2006; Jackson, 2008). This method of  
77 controlling invasives exposes large areas of bare ground, potentially influencing soil functioning and  
78 properties in many ways.

79 Firstly, the bare ground is exposed to increased solar radiation following canopy removal (Araujo and  
80 Austin, 2015). This would lead to increased soil temperature, and in turn, higher decomposition  
81 rates through enhanced microbial activity (Eliasson et al., 2005; Hartley et al., 2007; Rutledge et al.,  
82 2010). Increased solar radiation may also lead to greater photochemical breakdown of the  
83 recalcitrant litter compounds such as lignin and tannins, further enhancing the release of CO<sub>2</sub> from  
84 soil (Austin et al., 2016; Gliksman et al., 2018; Rutledge et al., 2010). Photodegradation, however,  
85 may not greatly influence soil carbon content, as UV radiation does not penetrate into the soil  
86 (Moorhead and Callaghan, 1994).

87 Canopy removal may also impact upon soil water content; increased surface air heat loss during the  
88 night post-clearance would lead to higher dew formation (Gliksman et al., 2018; Xiao et al., 2009)  
89 and potentially higher microbial activity as a result (Gliksman et al., 2017). Canopy interception of  
90 rainfall also results in less water reaching the ground (Llorens and Domingo, 2007), and its clearance  
91 will result in lower evapotranspiration. Removing the canopy may therefore result in higher soil  
92 moisture content, leading to higher microbial activity (Hanson et al., 2000).

93 Finally, cutting *R. ponticum* may change soil chemistry and microbial communities. A recent study in  
94 North America on another invasive shrub, *Lonicera maackii* (Rupr.) Herder, suggested that cutting  
95 leads to a pulse in rhizodeposition (Frank et al., 2018). Increased exudation of carbon substrates  
96 post-clearance influences the microbial community, in turn leading to altered SOM degradation

97 (Frank et al., 2018; Pignataro et al., 2012). Despite this, Osburn et al. (2018) found that the removal  
98 of a *Rhododendron maximum* canopy had no effect on the activity of enzymes involved in  
99 decomposition.

100 Invasive plant removal therefore has the potential to alter soil processes, chemical composition and  
101 microbial communities. Great emphasis is placed on removing invasive plants, given the critical role  
102 this plays in restoring native habitats and preventing further invasion (European Union, 2014).

103 However, our understanding of how these activities influence soil processes remains limited. As it  
104 will influence the vegetation community that can inhabit the soil post-clearance, and thus the  
105 success of restoration, increasing our understanding of the impacts of clearance on soil processes is  
106 important.

107 This study investigated the impact of *R. ponticum* invasion and its subsequent clearance on soil  
108 functioning and chemistry, testing four hypotheses. Firstly, that soil respiration is lower on uncleared  
109 plots of *R. ponticum* relative to adjacent native vegetation plots. Secondly, that clearing the canopy  
110 increases soil respiration, relative to uncleared *R. ponticum* plots. To provide a mechanistic  
111 explanation for the above hypotheses, it was further hypothesised that *R. ponticum* clearance would  
112 alter both (iii) soil microbial communities and (iv) SOM chemical composition.

## 113 **2. Materials and methods**

### 114 **2.1. Site description**

115 A 0.9 hectare site in Tanygrisiau (52°58'55" N 3°57'18" W), Snowdonia National Park, Wales, was  
116 selected for sampling due to the presence of well-established *R. ponticum* thickets (100% *R.*  
117 *ponticum* cover, <3 m tall). Uninvaded areas of native vegetation cover were also present, consisting  
118 of acid grassland species typical of the area including *Agrostis capillaris* L., *Nardus stricta* L., *Molinia*  
119 *caerulea* L., *Juncus effusus* L., *Carex echinata* Murray and to a lesser extent, shrubs such as *Calluna*  
120 *vulgaris* L. (Hull) and *Vaccinium myrtillus* L.. Soil at the site was a peaty podzol of the Hexworthy  
121 series (National Soil Resources Institute, 2019). The site has a north-east facing aspect with an

122 average gradient of 10%. Altitude at the area sampled varied from 180 m to 190 m. On average, the  
123 site received an annual 2678 mm of rain, whilst the mean air temperature was 9.3 °C over the five  
124 years prior to sampling (Met Office, 2018).

125 Between the 19<sup>th</sup> and 23<sup>rd</sup> of March 2018, eight 6 m x 20 m strips were cleared of *R. ponticum* by  
126 cutting the stems at their base and burning the material off-site; roots were not removed from the  
127 soil. These cleared strips alternated with uncleared 6 m wide strips (Figure S1). Subsequently, 3m ×  
128 3m plots were placed in the middle of the alternating cleared and uncleared strips (n = 8), with each  
129 plot surrounded by a 3 m buffer strip to avoid edge effects. Additionally, eight native vegetation  
130 plots were placed where *R. ponticum* had not invaded, as close as possible to the invaded plots  
131 whilst ensuring they were not influenced by *R. ponticum*. The experiment therefore had three plot  
132 types; cleared, native and uncleared.

## 133 **2.2. Soil respiration measurements**

134 Sampling was conducted before clearance on the 16<sup>th</sup> of March 2018. Five bulked soil cores (20 mm  
135 diameter) containing both O-horizon material and mineral soil were collected from each plot to a  
136 depth of 15 cm. Samples were freeze-dried (LTE Scientific Lyovac, Oldham, UK) and weighed to  
137 measure moisture content gravimetrically, then stored at -80 °C prior to chemical analyses. Soil  
138 respiration was measured on each plot between 10 am and 2 pm on three consecutive days to  
139 account for variation within timepoints, using an infra-red gas analyser (IRGA) (EGM-4, PP-systems,  
140 USA) connected to a soil chamber (SRC-1, PP-systems, USA). Alongside each soil respiration  
141 measurement, data for soil temperature (to a depth of 10 cm) (Hanson H2203A temperature probe),  
142 photosynthetically active radiation (PAR) (Skye Instruments PAR Special Sensor, Wales) and soil  
143 moisture (gravimetrically) were also collected.

144 To investigate the impact of *R. ponticum* canopy removal, the soil sampling and respiration  
145 measurements (and associated measurements) made at the start of the experiment were repeated  
146 six times over the following growing season (one, two, four, eight, 16- and 32-weeks post-clearance).

147 Soil samples collected both prior to clearance and 32-weeks post-clearance were analysed for their  
148 total carbon and nitrogen content with a Vario MAX cube analyser (Elementar, Langenselbold,  
149 Germany).

### 150 **2.3. Phospholipid fatty acid (PLFA) analysis**

151 Soil microbial community structure was investigated by analysis of PLFA profiles. Freeze-dried soil  
152 samples collected from each plot, both prior to and 32 weeks post *R. ponticum* removal, were sieved  
153 to remove roots and stones (2 mm mesh) and homogenised with a ball mill (Retsch MM200, Haan,  
154 Germany). PLFAs were analysed using a high throughput method adapted from Buyer and Sasser  
155 (2012), described in full detail in the supplementary information. Briefly, PLFAs were extracted from  
156 0.4-0.6 g of each freeze-dried soil sample using phosphate buffered Bligh and Dyer extractant.  
157 Freeze-dried extracts were subsequently dissolved in chloroform and transferred to solid phase  
158 extraction (SPE) plates (100 mg silica, Phenomenex, Torrance, CA, USA), to be eluted into a 96 well  
159 plate using acetone. Extracts were analysed using an Agilent 7890A gas chromatograph with a DB-  
160 5MS column and an FID detector.

161 In total, 16 PLFAs were identified in the soil samples. These included commonly used biomarkers of  
162 fungi (18:2(n-6,9), 18:2(n-6,9) and 18:1(n-9)) (Bååth and Anderson, 2003), Gram-positive bacteria  
163 (15:0i, 15:0a, 16:0i, i17:0 and a17:0) (Kidd Haack et al., 1994; Lechevalier and Lechevalier, 1988;  
164 Zelles, 1999) and Gram-negative bacteria (16:1, 7,cy-17:0, 18:1(n-7) and 7,8cy-19:0) (Galbraith and  
165 Wilkinson, 1991; Ratledge and Wilkinson, 1988; Zelles, 1999). These markers were used to measure  
166 the abundance of these microbial groups, as well as the fungi: bacteria (F:B) and Gram-positive:  
167 Gram-negative ratios (GP:GN) (Frostegård et al., 2011), which are indicators of substrate quality in  
168 soil (Fanin et al., 2019; Van Der Heijden et al., 2008).

### 169 **2.4. Fourier-transform mid-infrared spectroscopy (FTIR) analysis**

170 Soil chemistry was investigated by FTIR spectroscopy. Freeze-dried soil samples collected from each  
171 plot prior to clearance and 32 weeks post-clearance were sieved and milled, as previously described



172 above. Spectra were measured using an IRTracer-100 spectrophotometer (Shimadzu, Japan) fitted  
173 with a Golden Gate diamond ATR accessory (Specac Ltd., Orpington, UK). Absorption was recorded  
174 in duplicate with a wavelength range of 4000 to 600  $\text{cm}^{-1}$  and resolution of 2  $\text{cm}^{-1}$ . Between each  
175 sample, a background reading was taken to ensure that any atmospheric changes in  $\text{CO}_2$  and  $\text{H}_2\text{O}$   
176 were corrected for.

177 FTIR spectra showed several absorbance peaks in wavebands associated with bond vibrations in  
178 specific groups of compounds relevant for organic matter quality. These included peaks associated  
179 with C=C stretching (1620  $\text{cm}^{-1}$  and 1510  $\text{cm}^{-1}$ ) and C–O stretching (1420  $\text{cm}^{-1}$ ) in aromatic and  
180 carboxylic structures and C–O–C vibrations in polysaccharides (1020  $\text{cm}^{-1}$ ) (Artz et al., 2008;  
181 Haberhauer et al., 1998; Heller et al., 2015). Peak intensity for wavebands associated with aromatic  
182 compounds (sum of 1620  $\text{cm}^{-1}$ , 1510  $\text{cm}^{-1}$  and 1420  $\text{cm}^{-1}$ ) and polysaccharides (1020  $\text{cm}^{-1}$ ) was  
183 measured, whilst the aromaticity index was calculated as the ratio of absorbance in aromatic to  
184 polysaccharide wavebands (McAnallen et al., 2017).

## 185 **2.5. Statistical analyses**

186 All analyses were conducted using R statistical software (version 3.5.3) (R Development Core Team,  
187 2017). To test for differences among plots in soil respiration, generalised linear mixed models  
188 (GLMMs) were used for repeated measurements, whilst generalised linear models (GLMs) were used  
189 to analyse data within time points. Prior to analysis, we tested for normality and skewness,  
190 subsequently using appropriate error distributions and link functions in the models (see Table S1 for  
191 full details). Tukey's HSD test was used for pairwise comparisons, conducted using the *multcomp* R  
192 package. The soil respiration measurements made on three consecutive days were averaged, with  
193 the mean value used in statistical analyses. Soil temperature can influence soil respiration rates  
194 (Schaefer et al., 2009), and was therefore included as a covariate in models analysing soil respiration.  
195 Soil moisture was not included as a covariate as it did not improve model fit, following comparison  
196 of Akaike information criterion (AIC) values. Cumulative respiration was calculated as the area under

197 the curve, with the log-transformed data analysed by one-way ANOVA followed by Tukey's HSD test  
198 for pairwise comparisons. One-way ANOVA and Tukey's HSD test was also used to compare the  
199 abundances of specific PLFAs associated with different microbial groups among plot types. Prior to  
200 analysis, PLFA abundance data for each microbial group were normalized by the total PLFA  
201 abundance to obtain relative abundance, correcting for varying extraction efficiency between  
202 samples. Concentration data for each individual PLFA were Hellinger transformed, centred and  
203 scaled prior to cross-validated principal components analysis (PCA) (Legendre and Gallagher, 2001;  
204 Legendre and Legendre, 1998). To statistically test for differences in the PLFA profiles of different  
205 plots, Euclidean distance matrices were analysed by permutational multivariate ANOVA  
206 (PERMANOVA) with 10000 permutations, using the *adonis* package. FTIR spectral data were pre-  
207 processed by baseline correction and smoothing using the *ChemoSpec* package, whilst the 1800-  
208 2500  $\text{cm}^{-1}$  region was removed to minimise background variability. Scaled and centred spectral data  
209 were visualised by PCA, and Euclidean distance matrices were analysed by PERMANOVA as  
210 previously described. FTIR peak intensities for the specific wavebands and ratios previously  
211 described were analysed in GLMs and GLMMs.

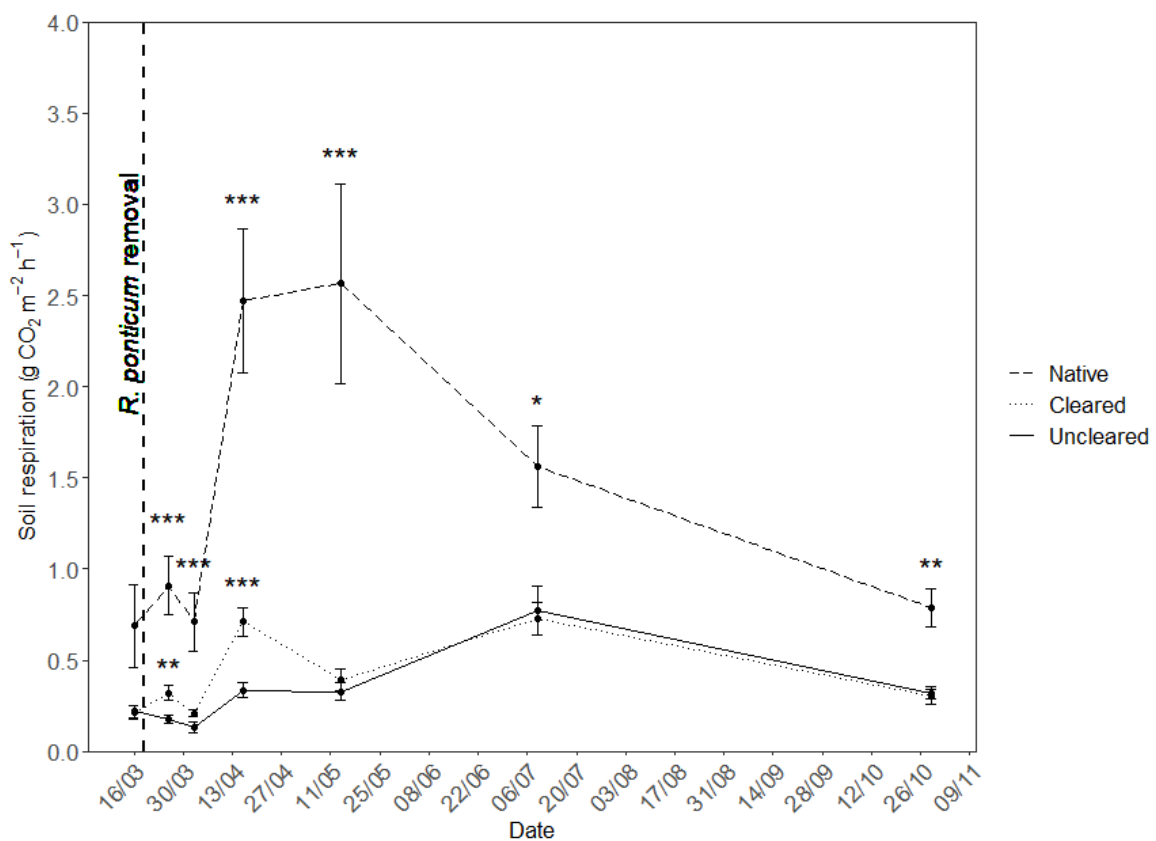
## 212 **3. Results**

### 213 **3.1. Impact of *R. ponticum* on soil respiration**

214 Over the whole growing season, soil respiration was significantly lower in the uncleared plots  
215 relative to the native vegetation plots ( $P < 0.001$ ) (Figure 1). Soil temperature significantly affected  
216 soil respiration when included as a covariate in the above model ( $P < 0.001$ ) and was significantly  
217 higher in native vegetation plots relative to uncleared plots over the growing season ( $P < 0.001$ )  
218 (Table S1). Soil moisture did not significantly differ among plot types ( $P = 0.170$ ), whilst PAR was  
219 significantly lower in uncleared plots ( $P < 0.001$ ) (Table S1).

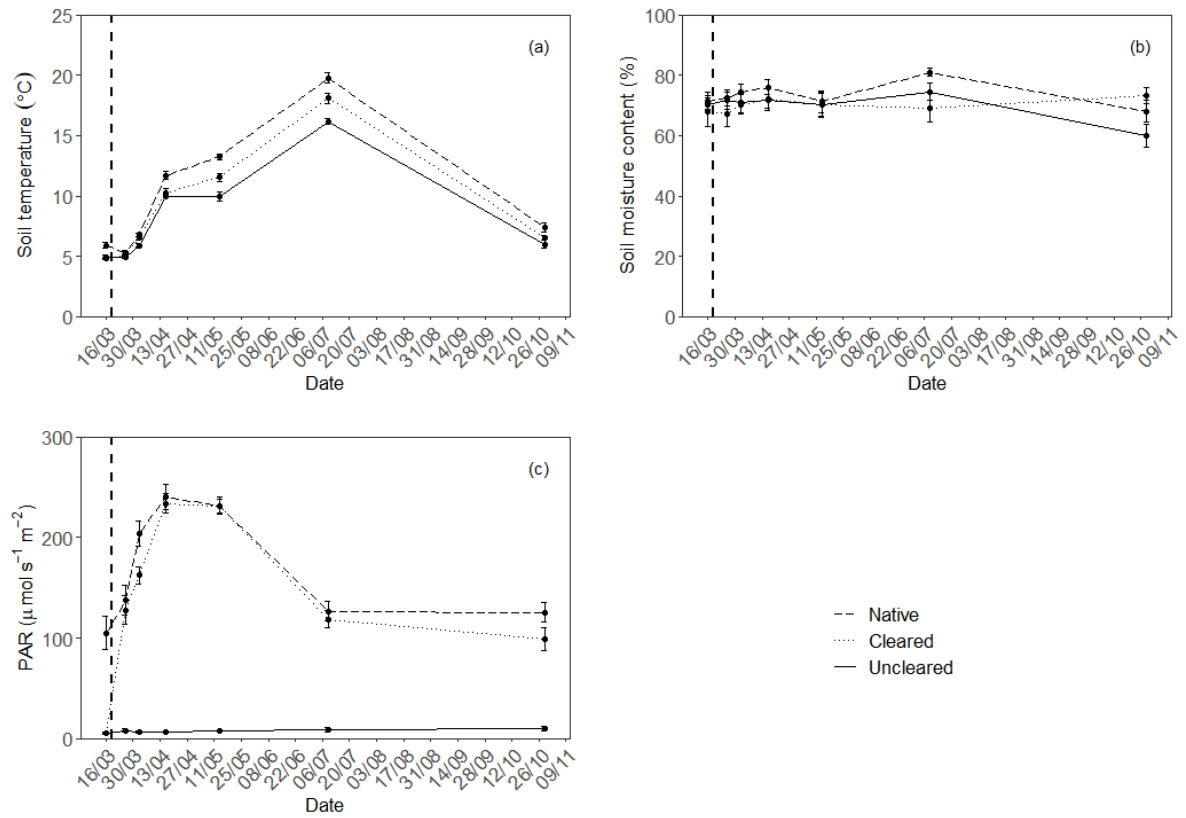
220 *R. ponticum* clearance did not increase soil respiration, with no significant difference in cumulative  
221 respiration between the cleared and uncleared plots over the growing season ( $P = 0.799$ ) (16.37 g

222 CO<sub>2</sub> m<sup>-2</sup> and 15.31 g CO<sub>2</sub> m<sup>-2</sup> respectively). Cumulative respiration during this period was significantly  
 223 higher on the native vegetation plots (50.17 g CO<sub>2</sub> m<sup>-2</sup>), relative to the cleared and uncleared plots ( $P$   
 224  $< 0.001$ ). Similarly, a repeated measures model detected no significant difference in soil respiration  
 225 between the cleared and uncleared plots over the 32-week period post-clearance ( $P = 0.214$ ) (Figure  
 226 1). Soil respiration in the native vegetation plots remained significantly higher than in the cleared  
 227 plots post *R. ponticum* removal over the same period ( $P < 0.001$ ) (Table S1). Repeated measures  
 228 analysis detected soil temperature was significantly higher in cleared plots relative to uncleared  
 229 plots over the 32 weeks post-clearance ( $P = 0.005$ ). When comparing plots within individual time  
 230 points, higher soil respiration on cleared plots relative to uncleared plots was observed one- and  
 231 four-weeks post-clearance ( $P = 0.009$  and  $P < 0.001$  respectively) (Figure 1). No differences were  
 232 observed between these plots two ( $P = 0.222$ ), eight ( $P = 0.619$ ), 16 ( $P = 0.783$ ) or 32 weeks ( $P =$   
 233 0.489) post-clearance.



235 **Figure 1:** Mean soil respiration ( $\pm$  standard error) in the cleared, native vegetation and uncleared  
236 plots over the 32-week duration of the experiment. Plots were cleared of *R. ponticum* during the  
237 week beginning 19<sup>th</sup> March 2018. Significantly higher soil respiration in the cleared or native plots  
238 relative to the uncleared plots is denoted by \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ) following  
239 analysis in GLMs.

240 Despite soil respiration being lower in cleared and uncleared plots relative to native vegetation  
241 plots, no significant differences were detected in the soil carbon percentage of these plots prior to  
242 clearance ( $P = 0.315$ ), or 32 weeks post-clearance ( $P = 0.105$ ) (Table 1). Similarly, soil nitrogen  
243 percentage did not vary significantly among plot types prior to clearance ( $P = 0.084$ ) (Table 1).  
244 Cleared plots had significantly lower soil nitrogen percentage compared to the native plots 32 weeks  
245 post-clearance ( $P = 0.004$ ), but not compared to the uncleared plots ( $P = 0.176$ ) (Table 1). No  
246 significant differences in soil nitrogen percentage were detected between the uncleared and the  
247 native plots post-clearance ( $P = 0.293$ ) (Table 1). The soil C:N ratio of the uncleared plots was  
248 significantly higher than the native vegetation plots at the start of experiment ( $P = 0.026$ ), however  
249 no significant differences were detected among any of the plot types 32 weeks post-clearance ( $P =$   
250  $0.139$ ). No significant differences in soil pH were observed among the different plot types either  
251 before or after clearance ( $P = 0.652$  and  $P = 0.749$  respectively) (Table 1).



252

253 **Figure 2:** Mean (a) soil temperature, (b) moisture content and (c) photosynthetically active radiation

254 (PAR) ( $\pm$  standard error) in the cleared, native vegetation and uncleared plots over the 32-week

255 duration of the experiment. The vertical dotted lines mark the time at which plots were cleared of *R.*

256 *ponticum* (during the week beginning 19<sup>th</sup> March 2018).

257 **Table 1:** Mean ( $\pm$  standard error) carbon content, nitrogen content, C:N ratio and pH of soils sampled from cleared, native vegetation or uncleared plots,  
 258 both before clearance and 32 weeks post-clearance. Common letters denote statistically non-significant differences ( $P > 0.05$ ) following analysis in GLMs.

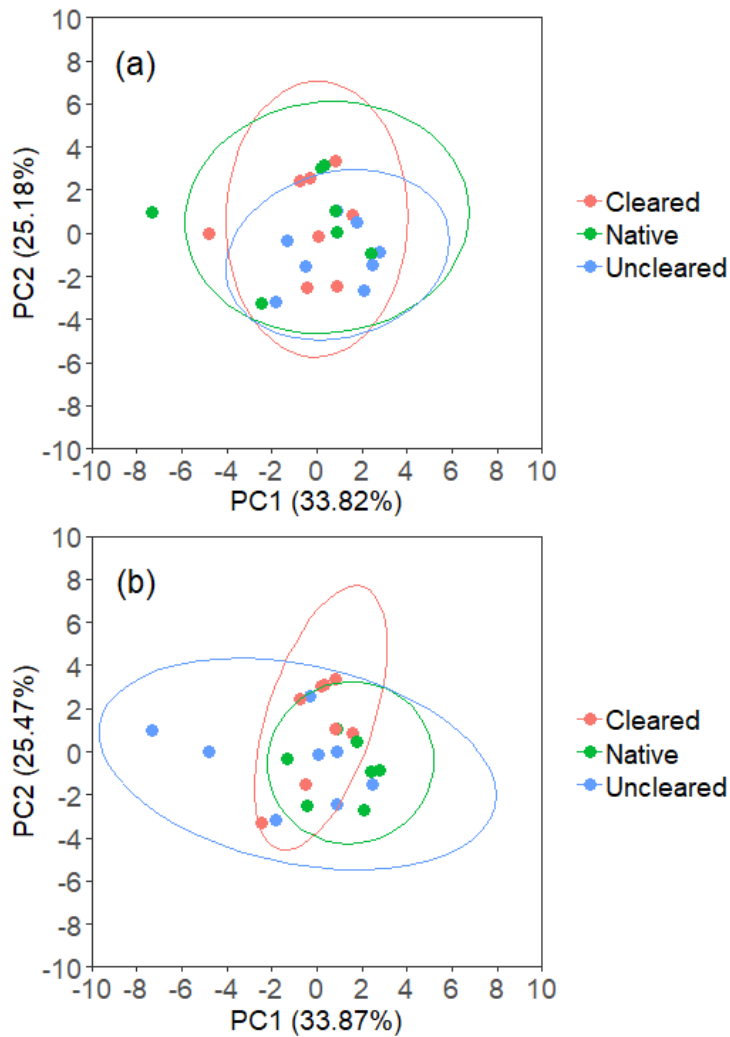
	Prior to clearance			32 weeks post-clearance		
	Cleared	Native	Uncleared	Cleared	Native	Uncleared
C content (%)	25.27 $\pm$ 3.44	32.47 $\pm$ 3.27	28.62 $\pm$ 2.91	29.23 $\pm$ 3.28	37.13 $\pm$ 2.14	34.44 $\pm$ 1.97
N content (%)	1.35 $\pm$ 0.19	1.91 $\pm$ 0.16	1.51 $\pm$ 0.17	1.52 $\pm$ 0.17 a	2.14 $\pm$ 0.10 b	1.86 $\pm$ 0.12 ab
C:N	18.70 $\pm$ 0.55 ab	16.88 $\pm$ 0.71 a	19.31 $\pm$ 0.71 b	19.26 $\pm$ 0.94	17.26 $\pm$ 0.55	18.61 $\pm$ 0.50
pH	4.03 $\pm$ 0.07	4.09 $\pm$ 0.09	3.97 $\pm$ 0.08	3.83 $\pm$ 0.09	3.85 $\pm$ 0.04	3.89 $\pm$ 0.08

259

### 260 3.2. Soil PLFA analysis

261 Hellinger transformed concentration data of 16 PLFAs extracted from soils collected prior to  
262 clearance and 32 weeks post-clearance were subjected to PCA. The first two PCs accounted for 59%  
263 of total variance prior to clearance, 59.34% post-clearance, and did not clearly separate the different  
264 plot types in either model (Figure 3). To statistically determine whether the clusters of each plot  
265 type were different, Euclidean distance matrices were analysed by PERMANOVA. These analyses  
266 corroborated the interpretation of the PCA, detecting no significant differences in PLFA profiles  
267 among the different plot types prior to clearance ( $P = 0.204$ ), or 32 weeks post-clearance ( $P = 0.066$ ).

268 No significant differences in total PLFA concentration were observed among the cleared, uncleared  
269 and native plots either prior to clearance ( $P = 0.727$ ), or 32 weeks post-clearance ( $P = 0.128$ ) (Table  
270 2). This was also true for the F:B and GP:GN ratios prior to clearance ( $P = 0.072$  and  $P = 0.942$   
271 respectively) and 32-weeks post-clearance ( $P = 0.201$  and  $P = 0.758$ ). Prior to clearance, no  
272 significant differences in Gram-negative ( $P = 0.262$ ) or total bacterial ( $P = 0.085$ ) PLFA abundance  
273 were observed among plots. However, the plots that were to be cleared had significantly lower  
274 fungal PLFA abundance ( $P = 0.038$ ) and higher Gram-positive abundance ( $P = 0.029$ ) compared to the  
275 plots that would be left uncleared. No significant differences in the relative abundances of the  
276 different microbial groups were observed among plots post-clearance.



277

278 **Figure 3:** Score plots for PCA models analysing the Hellinger transformed concentration data of the  
 279 16 PLFAs identified in the soil samples collected from the different plot types both (a) prior to *R.*  
 280 *ponticum* removal and (b) 32 weeks post-clearance. Plots were either cleared of *R. ponticum*, left  
 281 uncleared or uninvaded and consisting of native vegetation ( $n = 8$ ). Models were cross validated to  
 282 evaluate fit and avoid overfitting. Ellipses denote the 95% confidence intervals for each of the  
 283 different plot types.



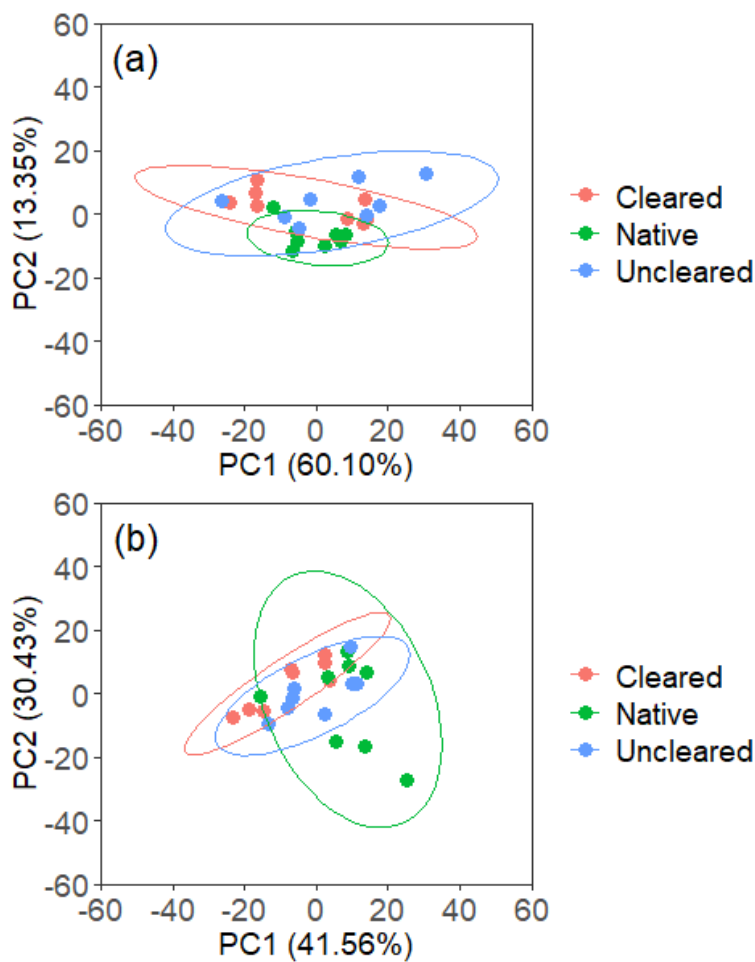
284 **Table 2:** Mean relative abundance (%) ( $\pm$  standard error) of PLFAs associated with different microbial groups in the samples collected from cleared, native  
 285 vegetation or uncleared plots, both before clearance and 32 weeks post-clearance. Total PLFA concentration ( $\text{nmol g}^{-1}$  soil) and the fungal: bacterial (F:B)  
 286 and Gram-positive: Gram-negative (GP:GN) ratios are also given. Common letters denote non-significant differences among the different plot types  
 287 following one-way ANOVA.

	Prior to clearance			32 weeks post-clearance		
	Cleared	Native	Uncleared	Cleared	Native	Uncleared
Fungal PLFAs (%)	44.14 $\pm$ 2.60 a	49.87 $\pm$ 1.80 ab	51.14 $\pm$ 0.99 b	51.89 $\pm$ 1.05	49.19 $\pm$ 1.26	50.48 $\pm$ 1.23
Bacterial PLFAs (%)	27.68 $\pm$ 1.58	25.04 $\pm$ 1.08	23.88 $\pm$ 0.66	26.65 $\pm$ 0.86	25.04 $\pm$ 0.98	21.92 $\pm$ 1.84
Gram-positive PLFAs (%)	14.25 $\pm$ 0.69 a	12.84 $\pm$ 0.52 ab	12.37 $\pm$ 0.25 b	11.86 $\pm$ 0.65	12.66 $\pm$ 0.60	11.25 $\pm$ 0.93
Gram-negative PLFAs (%)	13.02 $\pm$ 0.97	11.70 $\pm$ 0.71	11.17 $\pm$ 0.71	10.34 $\pm$ 0.41	11.91 $\pm$ 0.56	10.34 $\pm$ 0.11
Total PLFAs ( $\text{nmol g}^{-1}$ soil)	146.13 $\pm$ 17.13	163.89 $\pm$ 14.40	141.82 $\pm$ 27.72	186.63 $\pm$ 33.72	212.78 $\pm$ 38.89	124.56 $\pm$ 8.26
F:B	1.66 $\pm$ 0.18	2.04 $\pm$ 0.16	2.16 $\pm$ 0.10	2.32 $\pm$ 0.12	2.00 $\pm$ 0.12	2.45 $\pm$ 0.25
GP:GN	1.12 $\pm$ 0.05	1.11 $\pm$ 0.05	1.14 $\pm$ 0.09	1.15 $\pm$ 0.07	1.07 $\pm$ 0.05	1.14 $\pm$ 0.12

288

289 **3.3. Soil FTIR spectra analysis**

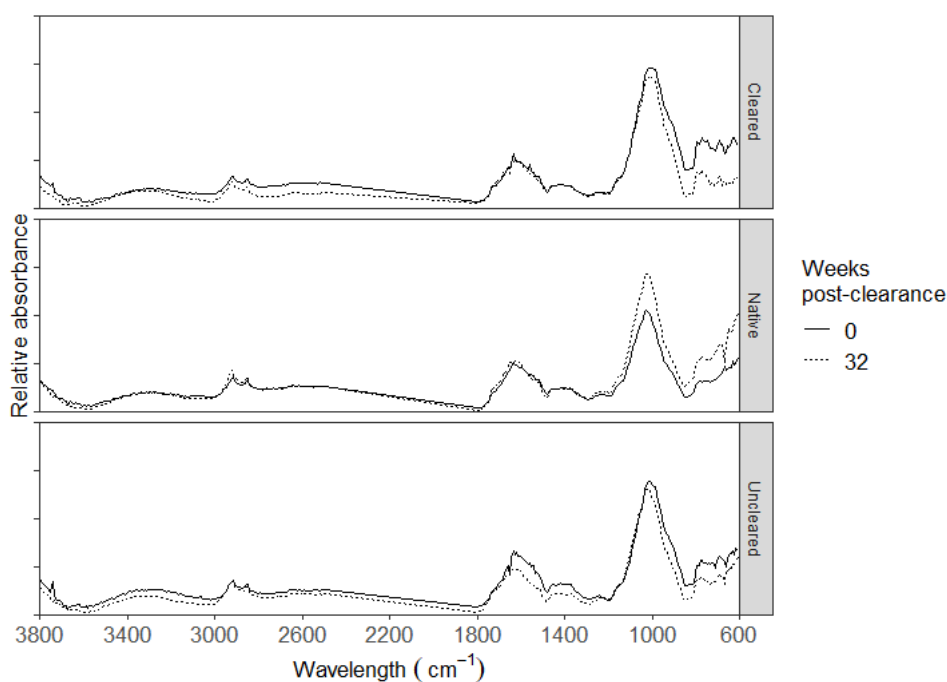
290 The first two PCs of soil samples collected from cleared, native vegetation and uncleared plots  
291 accounted for 73.45% of the total variance prior to clearance and 71.99% post-clearance. Partial  
292 separation by plot type was observed both prior to and 32 weeks post-clearance along PC1 and PC2  
293 (Figure 4). However, PERMANOVA detected no significant differences in Euclidean distances among  
294 the different plot types, either prior to- or 32 weeks post-clearance ( $P = 0.056$  and  $P = 0.122$   
295 respectively).



296

297 **Figure 4:** Score plots for PCA models analysing the FTIR spectra of soils collected from the different  
298 plot types both (a) prior to *R. ponticum* removal and (b) 32 weeks post-clearance. Plots were either  
299 cleared of *R. ponticum*, left uncleared or uninvaded and consisting of native vegetation ( $n = 8$ ).  
300 Models were cross validated to evaluate fit and avoid overfitting. Ellipses denote the 95% confidence  
301 intervals for each of the different plot types.

302 Visual inspection of FTIR spectra showed differences in peak intensities for specific wavebands  
303 relevant to SOM quality (Figure 5). At the start of the experiment, absorbance in the polysaccharide  
304 region was significantly lower for soil from native plots relative to the plots that would be cleared ( $P$   
305  $< 0.001$ ) and to the plots that would be left uncleared ( $P = 0.022$ ), whilst there was no difference  
306 between the cleared and uncleared plots ( $P = 0.541$ ) (Table 3). No differences among plot types  
307 were observed in this region post-clearance ( $P = 0.231$ ). Polysaccharide absorbance significantly  
308 increased for native plot soil over the 32-week growing season ( $P < 0.001$ ), whilst no significant  
309 change was observed for cleared and uncleared plots over the same period ( $P = 0.150$  and  $P = 0.917$   
310 respectively). Absorbance in the region associated with aromatic structures did not vary among plots  
311 in samples collected either prior to or post-clearance ( $P = 0.197$  and  $P = 0.894$  respectively).  
312 Aromatic region absorbance significantly decreased over the growing season for uncleared plot soil  
313 ( $P = 0.049$ ), whilst no significant change was observed for cleared ( $P = 0.846$ ) or native ( $P = 0.975$ )  
314 plot soil. The ratio of absorbance in aromatic to polysaccharide regions can be used as an index of  
315 aromaticity and humification (McAnallen et al., 2017). Soil aromaticity decreased over 32 weeks in  
316 both the native vegetation ( $P = 0.048$ ) and uncleared plots ( $P = 0.014$ ), however no change was  
317 observed for cleared plots ( $P = 0.957$ ).



319 **Figure 5:** Plot of FTIR spectra for soils sampled from plots cleared of *R. ponticum*, native vegetation  
 320 plots and plots left uncleared. Soils were sampled prior to (0 weeks) and 32 weeks post-clearance.

321 **Table 3:** Mean ( $\pm$  standard error) relative absorption (measured as peak intensity) of soil FTIR  
 322 spectra in wavebands associated with aromatic compounds ( $1620\text{ cm}^{-1}$ ,  $1510\text{ cm}^{-1}$  and  $1420\text{ cm}^{-1}$ ),  
 323 polysaccharides ( $1020\text{ cm}^{-1}$ ), and the ratio of absorbance in aromatic to polysaccharide wavebands  
 324 (aromaticity index). Soils were sampled both prior to and 32 weeks post *R. ponticum* clearance.  
 325 Common letters denote statistically non-significant differences within plots over time.

Plot type	Weeks post-clearance	Waveband relative absorption		
		Aromatics	Polysaccharides	Aromaticity index
Cleared	0	2.14 $\pm$ 0.36	2.55 $\pm$ 0.10	0.88 $\pm$ 0.18
	32	2.06 $\pm$ 0.26	2.39 $\pm$ 0.13	0.89 $\pm$ 0.13
Native	0	2.11 $\pm$ 0.16	1.96 $\pm$ 0.08 a	1.10 $\pm$ 0.11 a
	32	2.11 $\pm$ 0.24	2.63 $\pm$ 0.12 b	0.81 $\pm$ 0.10 b
Uncleared	0	2.76 $\pm$ 0.45 a	2.38 $\pm$ 0.14	1.15 $\pm$ 0.16 a
	32	1.95 $\pm$ 0.25 b	2.39 $\pm$ 0.06	0.81 $\pm$ 0.10 b

326 **4. Discussion**

327 Overall, soil respiration was significantly lower on uncleared *R. ponticum* plots relative to native  
 328 vegetation plots, consistent with the first hypothesis. Soil moisture and temperature are both known  
 329 to influence soil respiration rates (Hanson et al., 2000; Rutledge et al., 2010; Schaefer et al., 2009).  
 330 However, only soil temperature contributed towards the variation in soil respiration between  
 331 invaded and native vegetation plots in the current study, as soil moisture did not significantly vary  
 332 among plot types. Soil temperature was consistently higher in native vegetation plots than in  
 333 uncleared plots over the 32-week period monitored, most likely a consequence of the dense shade  
 334 cast by the *R. ponticum* canopy. Despite the observed differences in soil respiration, soil carbon  
 335 percentage did not vary between invaded and native plots. This may reflect the size of the soil

336 carbon pools, which are typically much larger than the amount of carbon released by soil respiration  
337 annually (Hartley et al., 2007; Valentini et al., 2000).

338 Leading from the lower soil respiration on uncleared plots relative to native vegetation plots, it was  
339 hypothesised that clearing *R. ponticum* would lead to increased soil respiration, relative to uncleared  
340 plots. Clearing led to a short-term increase in soil respiration at one- and four-weeks post-clearance.  
341 Several factors may have contributed towards this, including the observed increase in solar radiation  
342 at ground level post canopy removal, leading to higher soil temperature on cleared plots relative to  
343 uncleared plots, known to increase soil respiration (Rutledge et al., 2010; Yuste et al., 2004). Cutting  
344 woody shrubs and trees can also cause pulses in rhizodeposition and fine root mortality, which  
345 stimulate microbial activity and decomposition beneath the cut shrub (Frank et al., 2018; Pignataro  
346 et al., 2012).

347 The initial increase in soil respiration post-clearance did not persist over the whole growing season,  
348 despite the increase in soil temperature. This suggests that there were additional factors influencing  
349 soil respiration in cleared plot soil. Two explanations have previously been proposed for decreasing  
350 response in soil respiration to soil warming over time following perturbation (Hartley et al., 2007).

351 One potential reason is that the increase in soil temperature may cause a shift in soil microbial  
352 community structure, as different microbial groups have varying optimal temperature ranges  
353 (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997). The altered microbial community may have  
354 access to a greater carbon pool, as they are able to metabolise carbon substrates that were  
355 unavailable to the community before soil warming (Zogg et al., 1997). This initially results in higher  
356 soil respiration, before declining over time as the community acclimates to the warmer soil  
357 temperature (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997).

358 An altered microbial community is unlikely to explain the transient increase in soil respiration  
359 observed in the current study. Zogg et al. (1997) found shifts in PLFA profiles indicating altered  
360 microbial community structure in response to soil warming. However, we detected no significant

361 differences post-clearance among plot types in soil PLFAs, contrary to the third hypothesis, despite  
362 an increase in soil temperature post-clearance. The similar PLFA profiles of cleared, uncleared and  
363 native plot soils in the current study may reflect the low soil pH (pH of <4.1 for all plot types) and  
364 high C:N ratio of soil on the site, which tends to favour fungal dominated communities (Bååth and  
365 Anderson, 2003; Rousk et al., 2010). The PLFA method is regarded as an efficient and sensitive  
366 method of detecting shifts in the relative abundance of bacteria and fungi (Frostegård et al., 2011;  
367 Ramsey et al., 2006), and has detected shifts in previous studies on other invasives (Kourtev et al.,  
368 2003) and on soil warming (Zogg et al., 1997). Molecular techniques such as next generation  
369 sequencing may provide greater resolution for detecting finer changes in community composition,  
370 however Orwin et al. (2018) concluded that both approaches are broadly comparable. This is  
371 supported by the fact that no differences in soil F:B ratios were detected by Osburn et al. (2018)  
372 using a DNA sequencing technique two years post-clearance of the related *R. maximum*.  
373 Furthermore, the PLFA approach may be more suitable for detecting changes in higher taxonomic  
374 groups which lead to altered ecosystem functions (Orwin et al., 2018). We therefore consider the  
375 PLFA method to be appropriate for the current study.

376 Substrate limitation has also been suggested as a reason for declining soil respiration rates in  
377 response to increased soil temperature (Eliasson et al., 2005; Hartley et al., 2007). Soil warming  
378 initially leads to increased soil respiration, however microbial activity will decline over time as the  
379 pool of labile carbon becomes depleted (Eliasson et al., 2005; Hartley et al., 2007). This suggestion of  
380 substrate limitation is supported by the FTIR data, which is a commonly used technique to  
381 investigate shifts in SOM composition in a range of soil types (Elliott et al., 2007; Heller et al., 2015;  
382 McAnallen et al., 2017). Our fourth hypothesis which stated that soil FTIR spectra would be altered  
383 by clearing *R. ponticum* proved to be true; soil aromaticity was lower 32 weeks post-clearance  
384 (October 2018) compared to the start of the experiment (March 2018) in both the uncleared and  
385 native vegetation plots, whilst there was no change in this ratio in cleared plots during the same  
386 time period.

387 FTIR absorbance in regions associated with organic compounds may overlap with wavebands of  
388 mineral absorbance; for example  $\text{CaCO}_3$  absorbance may overlap with the  $1510\text{ cm}^{-1}$  and  $1620\text{ cm}^{-1}$   
389 wavebands associated with aromatic organic compounds, whilst kaolinite absorbance may overlap  
390 with the polysaccharide waveband ( $1020\text{ cm}^{-1}$ ) (Le Guillou et al., 2015). Despite this, we consider our  
391 approach to be appropriate given the peaty nature of the Hexworthy soil (National Soil Resources  
392 Institute, 2019) and the igneous bedrock (British Geological Society, 2019) present on the site. As a  
393 result, the calcareous content of the soil would be low, thus the effect of overlapping  $\text{CaCO}_3$   
394 absorbance is most likely negligible. Additionally, the low density of organic matter meant that by  
395 volume, the inorganic fraction of the soil was relatively low. Therefore, we argue that the soil  
396 organic fraction was the main influence on FTIR absorbance, and the overlapping of carbonates and  
397 clay minerals was likely to be negligible.

398 The fact that aromaticity decreased over the growing season for both uncleared and native plots,  
399 but not for the cleared plots suggests that *R. ponticum* removal influenced substrate quality. These  
400 observed variations in SOM quality may be due to changes in root exudation, canopy cover and litter  
401 deposition post-clearance. Whilst shrub clearance can lead to short-term pulses in rhizodeposition  
402 (Frank et al., 2018), canopy removal will result in an absence of photosynthesis in cleared plots, and  
403 thus lower photosynthate transfer to the soil in the longer-term, relative to uncleared and native  
404 vegetation plots. Additionally, carbon substrates are also introduced to the soil from canopy  
405 throughfall and in leachates from freshly deposited leaf litter (Frank et al., 2018; Hättenschwiler and  
406 Vitousek, 2000). The bare soil of the cleared plots would therefore likely have received lower input  
407 of fresh carbon substrates post removal of the *R. ponticum* canopy, relative to the soil of the  
408 uncleared and native plots. Thus, the pool of labile polysaccharides in the cleared plot soil would  
409 become depleted relative to the aromatic compounds, which are more resistant to decomposition  
410 and therefore become enriched in the soil (McAnallen et al., 2017; von Lützow et al., 2006).

411 Whilst soil aromaticity decreased for the uncleared plots and remained stable for the cleared plots  
412 over the experimental period, soil respiration did not differ at the end of this period between  
413 cleared and uncleared plots. This suggests that there were additional contributory factors  
414 influencing SOM decomposition in addition to substrate recalcitrance. Seasonal variation in soil  
415 temperature may be important, with the differences between cleared and uncleared plots becoming  
416 less pronounced in the Autumn. Soil physicochemical and biological factors are also known to  
417 influence decomposition, through organic matter stabilisation (Schmidt et al., 2011; von Lützow et  
418 al., 2006). Soil microbes may transform labile carbon substrates to stable compounds, which are  
419 more resistant to decomposition (Cotrufo et al., 2013; Schmidt et al., 2011). Carbon substrates may  
420 also adsorb to mineral surfaces or be physically occluded within soil aggregates, thus making them  
421 unavailable to decomposer organisms (Cotrufo et al., 2013; Schmidt et al., 2011; Zimmermann et al.,  
422 2012). Furthermore, carbon substrate availability to decomposer organisms can also be limited by  
423 binding to polyphenols (Bending and Read, 1997; Six et al., 2002). This may be particularly important  
424 for *R. ponticum*, as tannins leaching from the litter of the related *R. maximum* are reported to form  
425 decay-resistant complexes with SOM, leading to slower mineralisation rates (Wurzburger and  
426 Hendrick, 2009, 2007).

427 The lack of soil respiration response observed post-clearance in the current study may therefore be  
428 explained by substrate recalcitrance and SOM stabilisation. Differences in soil respiration remained  
429 between the cleared and native plots after one growing season. The persistence of this difference  
430 will likely depend on the revegetation of the bare soil left behind post-clearance, and a recent study  
431 highlighted that a native vegetation cover can take up to eight years to fully restore post *R. ponticum*  
432 removal (Jones et al., 2019). Therefore, to investigate whether soil processes and functioning in  
433 cleared plots transition towards native conditions post-clearance, a decadal study may be needed.



## 434 **5. Conclusions**

435 Our results show a short-term pulse in in soil respiration when *R. ponticum* is removed. This  
436 occurred during the initial four weeks post-clearance and was a response to higher soil temperature,  
437 but was limited in the longer-term by SOM quality. Soil PLFA profiles did not vary among cleared,  
438 native and uncleared plots post-clearance, indicating that *R. ponticum* removal did not alter the  
439 biomasses of different microbial groups. Our findings underline the strong influence of aboveground  
440 vegetation on soil respiration. Soil processes in the longer-term post-clearance will depend on the  
441 regeneration of native plant communities with cleared soil potentially taking decades to return to  
442 typical natural conditions.

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