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

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Abstract

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

Keywords: “Invasive”; “soil”; “respiration”; “decomposition”; “PLFAs”; “FTIR”; “soil organic matter”.

1. Introduction

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

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

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

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

Firstly, the bare ground is exposed to increased solar radiation following canopy removal (Araujo and Austin, 2015). This would lead to increased soil temperature, and in turn, higher decomposition rates through enhanced microbial activity (Eliasson et al., 2005; Hartley et al., 2007; Rutledge et al., 2010). Increased solar radiation may also lead to greater photochemical breakdown of the recalcitrant litter compounds such as lignin and tannins, further enhancing the release of CO_2 from soil (Austin et al., 2016; Gliksman et al., 2018; Rutledge et al., 2010). Photodegradation, however, may not greatly influence soil carbon content, as UV radiation does not penetrate into the soil (Moorhead and Callaghan, 1994).

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

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

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

Invasive plant removal therefore has the potential to alter soil processes, chemical composition and microbial communities. Great emphasis is placed on removing invasive plants, given the critical role this plays in restoring native habitats and preventing further invasion (European Union, 2014). However, our understanding of how these activities influence soil processes remains limited. As it will influence the vegetation community that can inhabit the soil post-clearance, and thus the success of restoration, increasing our understanding of the impacts of clearance on soil processes is important.

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

2. Materials and methods

2.1. Site description

A 0.9 hectare site in Tanygrisiau (52°58’55” N 3°57’18” W), Snowdonia National Park, Wales, was selected for sampling due to the presence of well-established *R. ponticum* thickets (100% *R. ponticum* cover, <3 m tall). Uninvaded areas of native vegetation cover were also present, consisting of acid grassland species typical of the area including *Agrostis capillaris* L., *Nardus stricta* L., *Molinia caerulea* L., *Juncus effuses* L., *Carex echinata* Murray and to a lesser extent, shrubs such as *Calluna vulgaris* L. (Hull) and *Vaccinium myrtillus* L.. Soil at the site was a peaty podzol of the Hexworthy 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 ^{o}C over the five
124 years prior to sampling (Met Office, 2018).

125 Between the 19th and 23rd 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 16th 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 ^{o}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).

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

2.3. Phospholipid fatty acid (PLFA) analysis

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

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

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

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

above. Spectra were measured using an IRTracer-100 spectrophotometer (Shimadzu, Japan) fitted with a Golden Gate diamond ATR accessory (Specac Ltd., Orpington, UK). Absorption was recorded in duplicate with a wavelength range of 4000 to 600 cm^{-1} and resolution of 2 cm^{-1}. Between each sample, a background reading was taken to ensure that any atmospheric changes in CO_2 and H_2O were corrected for.

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

2.5. Statistical analyses

All analyses were conducted using R statistical software (version 3.5.3) (R Development Core Team, 2017). To test for differences among plots in soil respiration, generalised linear mixed models (GLMMs) were used for repeated measurements, whilst generalised linear models (GLMs) were used to analyse data within time points. Prior to analysis, we tested for normality and skewness, subsequently using appropriate error distributions and link functions in the models (see Table S1 for full details). Tukey's HSD test was used for pairwise comparisons, conducted using the *multcomp* R package. The soil respiration measurements made on three consecutive days were averaged, with the mean value used in statistical analyses. Soil temperature can influence soil respiration rates (Schaefer et al., 2009), and was therefore included as a covariate in models analysing soil respiration. Soil moisture was not included as a covariate as it did not improve model fit, following comparison 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 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_2 m^{-2} and 15.31 g CO_2 m^{-2} respectively). Cumulative respiration during this period was significantly
223 higher on the native vegetation plots (50.17 g CO_2 m^{-2}), 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.

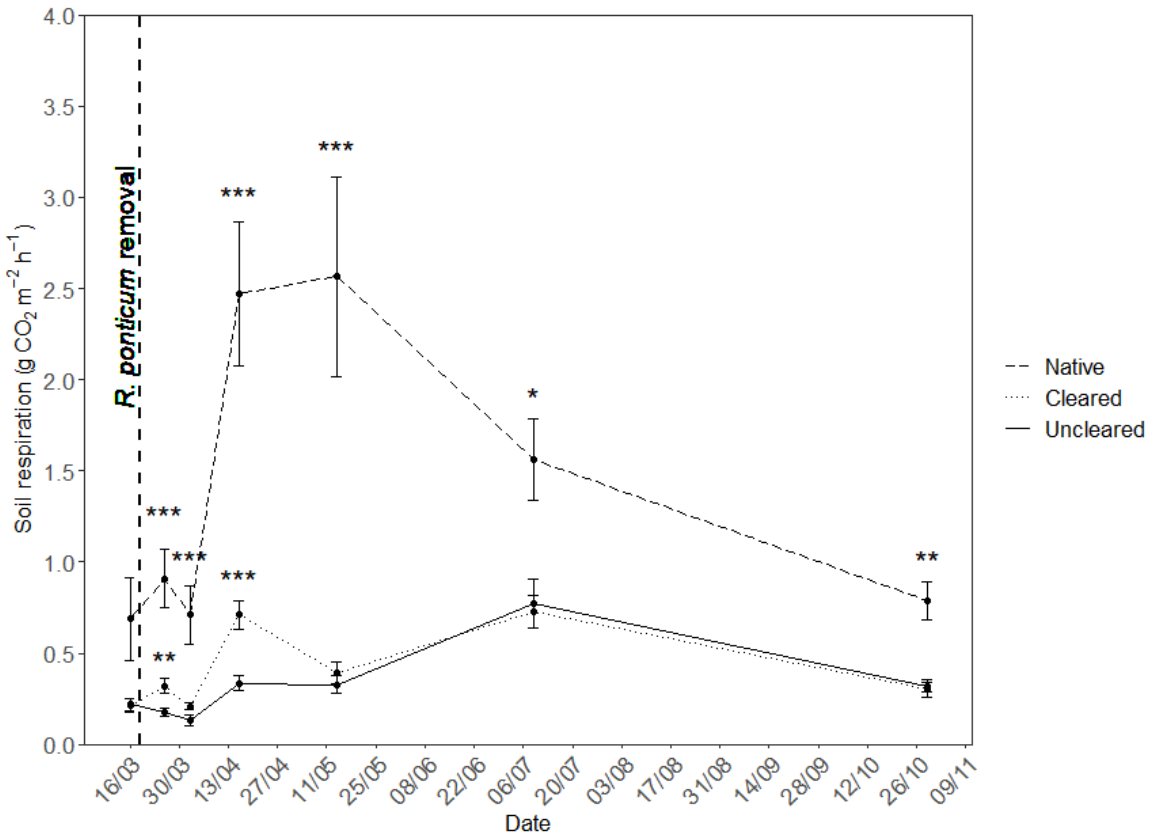

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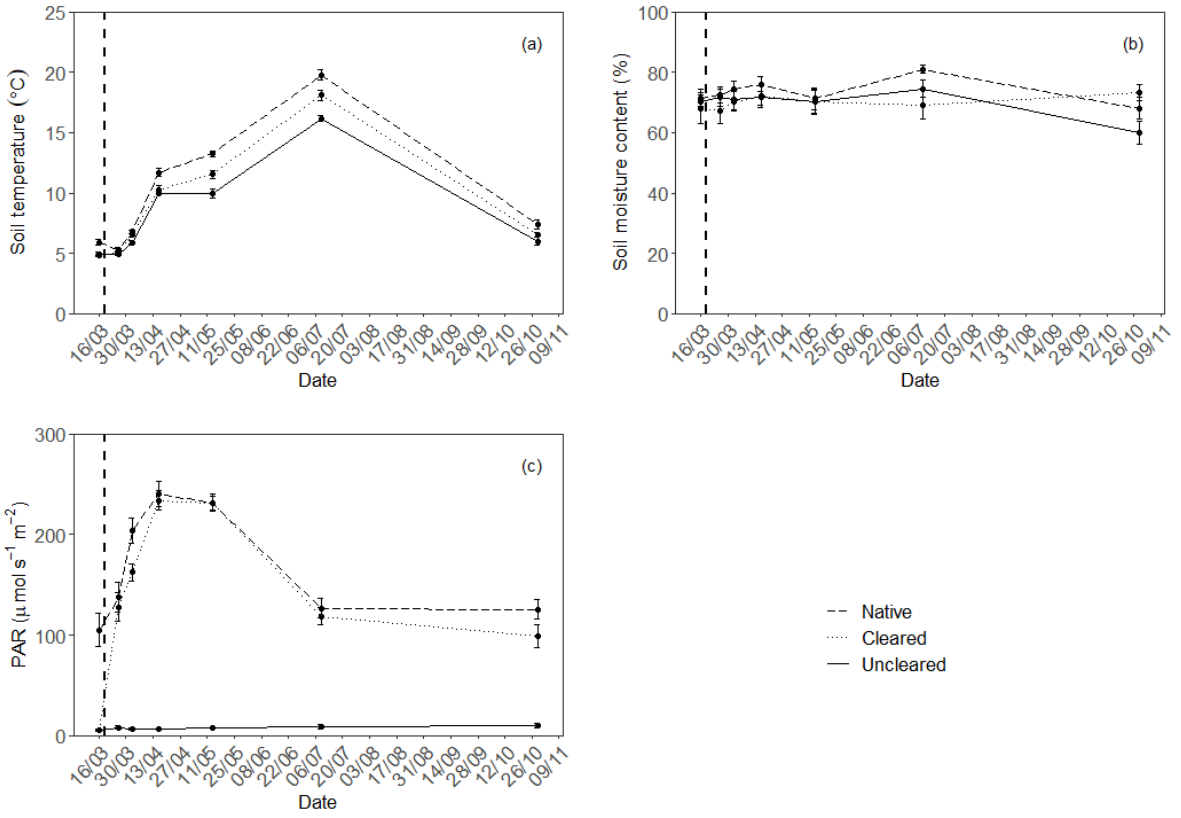
Figure 1: Mean soil respiration (± standard error) in the cleared, native vegetation and uncleared plots over the 32-week duration of the experiment. Plots were cleared of *R. ponticum* during the week beginning 19th March 2018. Significantly higher soil respiration in the cleared or native plots relative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

Despite soil respiration being lower in cleared and uncleared plots relative to native vegetation plots, no significant differences were detected in the soil carbon percentage of these plots prior to clearance ($P = 0.315$), or 32 weeks post-clearance ($P = 0.105$) (Table 1). Similarly, soil nitrogen percentage did not vary significantly among plot types prior to clearance ($P = 0.084$) (Table 1). Cleared plots had significantly lower soil nitrogen percentage compared to the native plots 32 weeks post-clearance ($P = 0.004$), but not compared to the uncleared plots ($P = 0.176$) (Table 1). No significant differences in soil nitrogen percentage were detected between the uncleared and the native plots post-clearance ($P = 0.293$) (Table 1). The soil C:N ratio of the uncleared plots was significantly higher than the native vegetation plots at the start of experiment ($P = 0.026$), however no significant differences were detected among any of the plot types 32 weeks post-clearance ($P = 0.139$). No significant differences in soil pH were observed among the different plot types either 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) (± 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 19th March 2018).

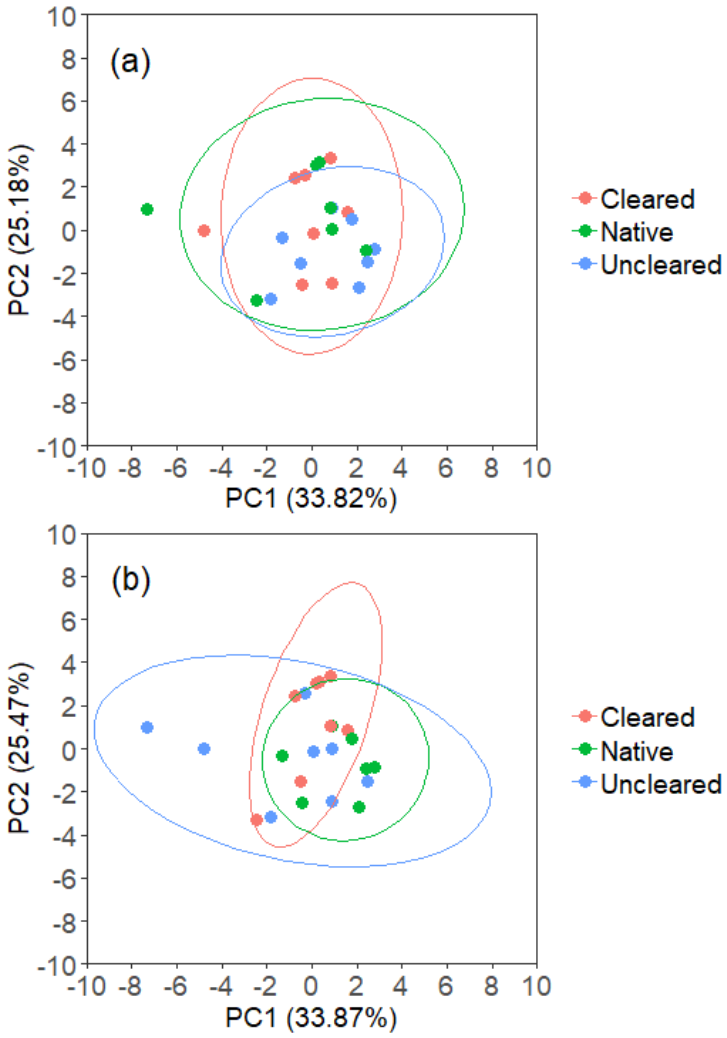
Table 1: Mean (± standard error) carbon content, nitrogen content, C:N ratio and pH of soils sampled from cleared, native vegetation or uncleared plots, both before clearance and 32 weeks post-clearance. Common letters denote statistically non-significant differences ($P > 0.05$) following analysis in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

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

3.2. Soil PLFA analysis

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

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



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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.

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

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

3.3. Soil FTIR spectra analysis

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

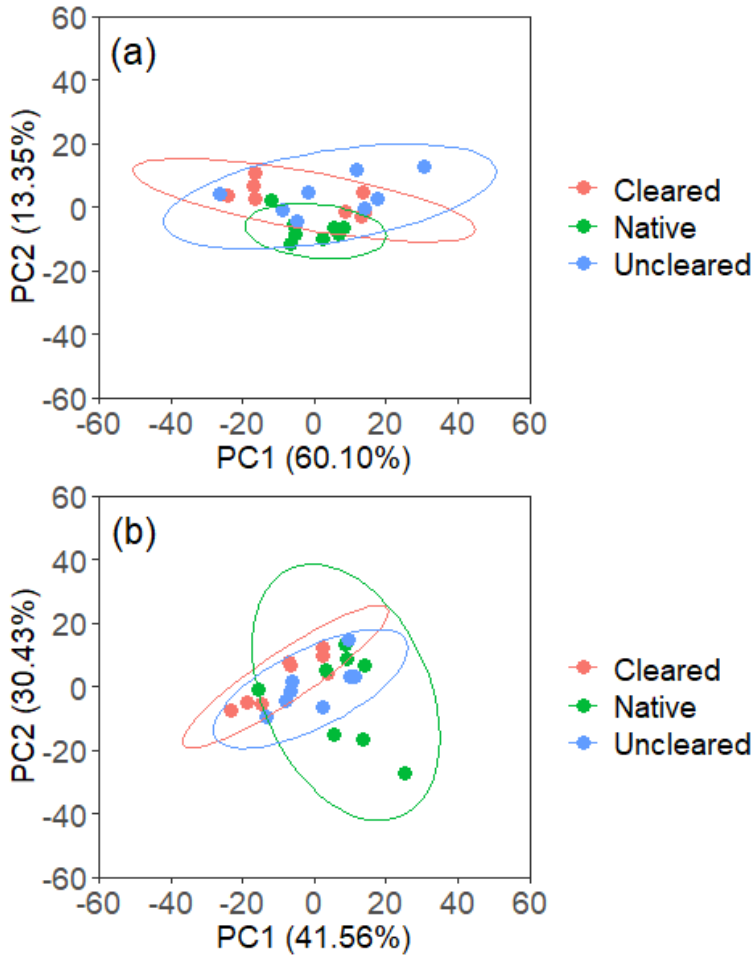

Figure 4: Score plots for PCA models analysing the FTIR spectra of soils collected from the different plot types both (a) prior to *R. ponticum* removal and (b) 32 weeks post-clearance. Plots were either cleared of *R. ponticum*, left uncleared or uninvaded and consisting of native vegetation (n = 8).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

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

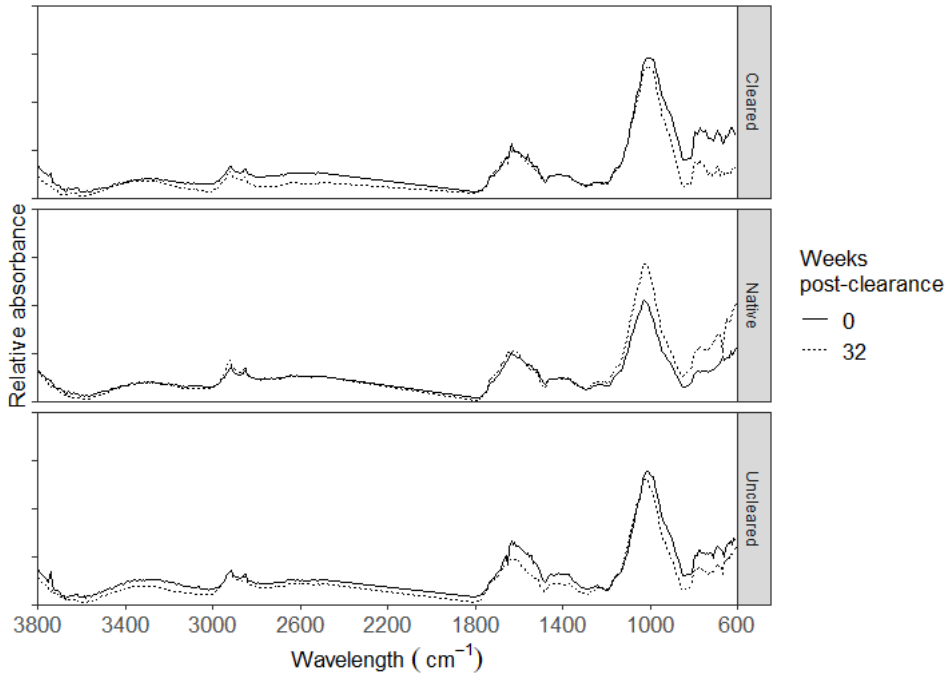

Figure 5: Plot of FTIR spectra for soils sampled from plots cleared of *R. ponticum*, native vegetation plots and plots left uncleared. Soils were sampled prior to (0 weeks) and 32 weeks post-clearance.

Table 3: Mean (± standard error) relative absorption (measured as peak intensity) of soil FTIR spectra in wavebands associated with aromatic compounds (1620 cm^{-1}, 1510 cm^{-1} and 1420 cm^{-1}), polysaccharides (1020 cm^{-1}), and the ratio of absorbance in aromatic to polysaccharide wavebands (aromaticity index). Soils were sampled both prior to and 32 weeks post *R. ponticum* clearance. 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 ± 0.36	2.55 ± 0.10	0.88 ± 0.18
	32	2.06 ± 0.26	2.39 ± 0.13	0.89 ± 0.13
Native	0	2.11 ± 0.16	1.96 ± 0.08 a	1.10 ± 0.11 a
	32	2.11 ± 0.24	2.63 ± 0.12 b	0.81 ± 0.10 b
Uncleared	0	2.76 ± 0.45 a	2.38 ± 0.14	1.15 ± 0.16 a
	32	1.95 ± 0.25 b	2.39 ± 0.06	0.81 ± 0.10 b

4. Discussion

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

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

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

The initial increase in soil respiration post-clearance did not persist over the whole growing season, despite the increase in soil temperature. This suggests that there were additional factors influencing soil respiration in cleared plot soil. Two explanations have previously been proposed for decreasing response in soil respiration to soil warming over time following perturbation (Hartley et al., 2007). One potential reason is that the increase in soil temperature may cause a shift in soil microbial community structure, as different microbial groups have varying optimal temperature ranges (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997). The altered microbial community may have access to a greater carbon pool, as they are able to metabolise carbon substrates that were unavailable to the community before soil warming (Zogg et al., 1997). This initially results in higher soil respiration, before declining over time as the community acclimates to the warmer soil temperature (Hartley et al., 2007; Luo et al., 2001; Zogg et al., 1997).

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

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

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

FTIR absorbance in regions associated with organic compounds may overlap with wavebands of mineral absorbance; for example $CaCO_3$ absorbance may overlap with the 1510 cm^{-1} and 1620 cm^{-1} wavebands associated with aromatic organic compounds, whilst kaolinite absorbance may overlap with the polysaccharide waveband (1020 cm^{-1}) (Le Guillou et al., 2015). Despite this, we consider our approach to be appropriate given the peaty nature of the Hexworthy soil (National Soil Resources Institute, 2019) and the igneous bedrock (British Geological Society, 2019) present on the site. As a result, the calcareous content of the soil would be low, thus the effect of overlapping $CaCO_3$ absorbance is most likely negligible. Additionally, the low density of organic matter meant that by volume, the inorganic fraction of the soil was relatively low. Therefore, we argue that the soil organic fraction was the main influence on FTIR absorbance, and the overlapping of carbonates and clay minerals was likely to be negligible.

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

5. Conclusions

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

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