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Long-term effects of forest fires on soil greenhouse gas emissions and extracellular enzyme activities in a hemiboreal forest

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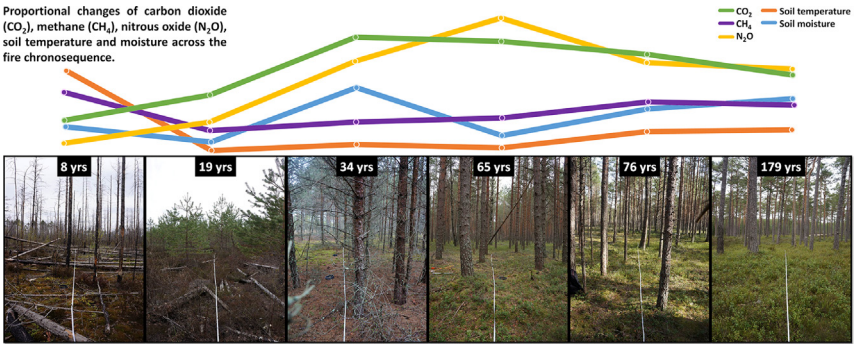
HIGHLIGHTS

- Soil respiration responded differently to soil temperature across the chronosequence.
- Methane sink and nitrous oxide emissions increased with soil temperature.
- Living root biomass only significantly increased 34 years after fire.
- Litter decomposition 8 years after fire was slower than pre-fire.
- Carbon-targeting enzymes did not significantly change across the chronosequence.

GRAPHICAL ABSTRACT



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ABSTRACT

Fire is the most important natural disturbance in boreal forests, and it has a major role regulating the carbon (C) budget of these systems. With the expected increase in fire frequency, the greenhouse gas (GHG) budget of boreal forest soils may change. In order to understand the long-term nature of the soil–atmosphere GHG exchange after fire, we established a fire chronosequence representing successional stages at 8, 19, 34, 65, 76 and 179 years following stand-replacing fires in hemiboreal Scots pine forests in Estonia. Changes in extracellular activity, litter decomposition, vegetation biomass, and soil physicochemical properties were assessed in relation to carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions. Soil temperature was highest 8 years after fire, whereas soil moisture varied through the fire chronosequences without a consistent pattern. Litter decomposition and CO_2 efflux were still lower 8 years after fire compared with pre-fire levels (179 years after fire). Both returned to pre-fire levels before vegetation re-established, and CO_2 efflux was only strongly responsive to temperature from 19 years after fire onward. Recovery of CO_2 efflux in the long term was associated with a moderate effect of fire on enzyme activity, the input of above- and below-ground litter carbon, and the re-establishment of vegetation. Soil acted as a CH_4 sink and N_2O source similarly in all successional stages. Compared with soil moisture and time after fire, soil temperature was the most important predictor for both GHGs. The

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re-establishment of overstorey and vegetation cover (mosses and lichens) might have caused an increase in CH_4 and N_2O effluxes in the studied areas, respectively.

1. Introduction

Forests and soils of the northern hemisphere account for most of the net forest carbon (C) sink in the world (Pan et al., 2011), hence their importance in tackling climate change. However, a projected increase in forest disturbances (e.g. wildfires, strong winds, insect outbreaks) may reduce forest C stocks (Seidl et al., 2014). Wildfires are projected to increase worldwide within the coming decades as part of a global warming trend (Pechony and Shindell, 2010). The northeastern region of Europe is among the most responsive areas to climate change (Giorgi, 2006). This region comprises coniferous and deciduous tree mixtures characterizing a transition zone between temperate and boreal forest termed the "hemiboreal zone" (Nilsson, 1997). These forests are more distinctively present at the southern part of the Eurasian boreal forest (Chytrý et al., 2008), especially in the Baltic region (Jõgiste et al., 2017) and to some extent in the southernmost part of Norway, Sweden and Finland (Olson et al., 2001). An increase in spring droughts during the next 100 years is expected in the Scandinavian and Baltic countries (Spinoni et al., 2018). Moreover, snowmelt and spring recovery of C uptake in the boreal zone already occurs 8 days earlier than 40 years ago (Pulliainen et al., 2017). Dry weather coupled with increased fuel availability could further lengthen the forest fire season, which has globally increased by nearly 20% during the past decades (Jolly et al., 2015).

During fire, the above- and below-ground plant biomass, humus layer and soil organic matter (SOM) can be combusted, releasing greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Shorohova et al., 2009; Yarie and Billings, 2002). Soil GHG production and consumption may also be altered in fire-affected areas as a result of changes in physical, chemical and biological soil properties during the fire succession (Certini, 2005; Hart et al., 2005; O'Neill et al., 2002). Soils are a considerable source of CO_2, exceeding anthropogenic fossil fuel combustion (Bond-Lamberty and Thomson, 2010). Total soil CO_2 efflux comprises autotrophic (i.e. plant roots and bryophytes) and heterotrophic (i.e. bacteria, fungi and soil fauna) respiration (Edwards et al., 1973), which results from the decomposition of above- and below-ground litter, and SOM (Wayson et al., 2006). Microbial biomass, litter decomposition, enzyme activity assays, and soil organic C, all provide insights into changes in SOM, which represents one of the greatest impacts of fire on soils (Certini, 2005) and generally results in a reduction of soil respiration (Luo and Zhou, 2006). Gupta and Mackenzie (2016) observed a negative effect of enzyme activity on soil respiration after fire, possibly due to fire-induced changes in C substrate availability. This discovery indicates the importance of considering the effect of fire on the interaction between biotic and abiotic processes related to decomposition when predicting CO_2 emissions during a fire succession.

Mineral soils of boreal forests are on average CH_4 sinks (Dalal and Allen, 2008). However, increasing forest fire frequency and intensity may cause changes in these patterns (Castaldi and Fierro, 2005). During the smouldering phase of combustion, a pulse of CH_4 is released to the atmosphere (Dalal and Allen, 2008; Hao and Ward, 1993). After fire, the nature of the soil–atmosphere exchanges of CH_4 is less obvious, as fire-induced changes to soil properties, such as moisture and temperature, and the time since fire affect the direction and rate of gas exchange (Burke et al., 2004; Nakano, 2006; Sullivan et al., 2011). In the short term (<1 year after fire), boreal forests affected by fires were reported to take up less CH_4 compared with unburned forests (Burke et al., 2004; Kulmala et al., 2014). However, after the initial decades post-fire, the uptake trend is less clear, with fluxes surpassing pre-fire levels (Köster et al., 2015a) or not changing at all (Köster et al., 2018).

Soil emissions of N_2O result from microbially-mediated denitrification and nitrification processes (Ussiri and Lal, 2013), although some fungi are also able to denitrify (Shoun et al., 1992). As *in situ* quantifications of N_2O uptake are still uncertain, it is possible that both nitrification and denitrification processes lead to N_2O uptake as well (Chapuis-Lardy et al., 2007). Despite being often neglected, net negative N_2O fluxes have been reported for northern forests (Castro et al., 1992; Klemedtsson et al., 1997). The processes involved in soil N_2O uptake are not clear yet, but many of their driving factors (e.g. availability of labile C and nitrogen (N), soil moisture, temperature and pH) (Chapuis-Lardy et al., 2007) are soil properties significantly affected by fires (Certini, 2005).

Wildfire suppression and management has almost eliminated fire occurrence in the hemiboreal zone during the last century (Nilsson, 1997), which has limited fire research conducted in these areas. However, climate change anticipated effects on fire regime metrics may lead to increased fire activity in the near future (Coogan et al., 2019), challenging not only fire suppression techniques, but also our understanding of feedbacks that link forest soils, fire and climate. Therefore, the aim of our study was to understand the long-term nature of the soil–atmosphere gas exchange following stand-replacing wildfires in hemiboreal forests of Estonia. Our specific objectives were: i) to assess fire-induced changes in soil GHG fluxes, plant biomass, and in physical, chemical and biological soil properties in each successional stage, ii) to quantify the relationship between soil GHG fluxes and soil temperature and moisture for different successional stages, and iii) to explore additional mechanisms underlying soil GHG fluxes along the fire succession. The effects of fire have been found to limit microbiological decay of litter and SOM (Holden et al., 2013). Moreover, besides temporally controlling soil CO_2 efflux (Saiz et al., 2006), soil temperature and moisture interact with the microbiota affecting also CH_4 and N_2O soil fluxes (Oertel et al., 2016). Thus, we hypothesized that fire-induced effects on soil temperature and moisture will affect fluxes of CO_2, CH_4 and N_2O differently across the successional stages. We also expected that post-fire changes in enzyme activity and litter mass loss will reflect the initial reduction in CO_2 fluxes.

2. Materials and methods

2.1. Study site

We selected six forest stands from a hemiboreal vegetation zone in Vihterpalu and Nõva, in northwestern Estonia, to study the effects of forest fires along a fire chronosequence. All the stands were flat with no elevation differences, located within a 145 km^2 area (Köster et al., 2016), and represented hemiboreal ecosystems at 8- (59°11′N 23°46′E), 19- (59°12′N 23°49′E), 34- (59°12′N 23°48′E), 65- (59°14′N 23°49′E), 76- (59°10′N 23°42′E) and 179-year (59°13′N 23°36′E) age classes of successional stages following stand-replacing fires (Fig. 1). Each age class comprised three sam-

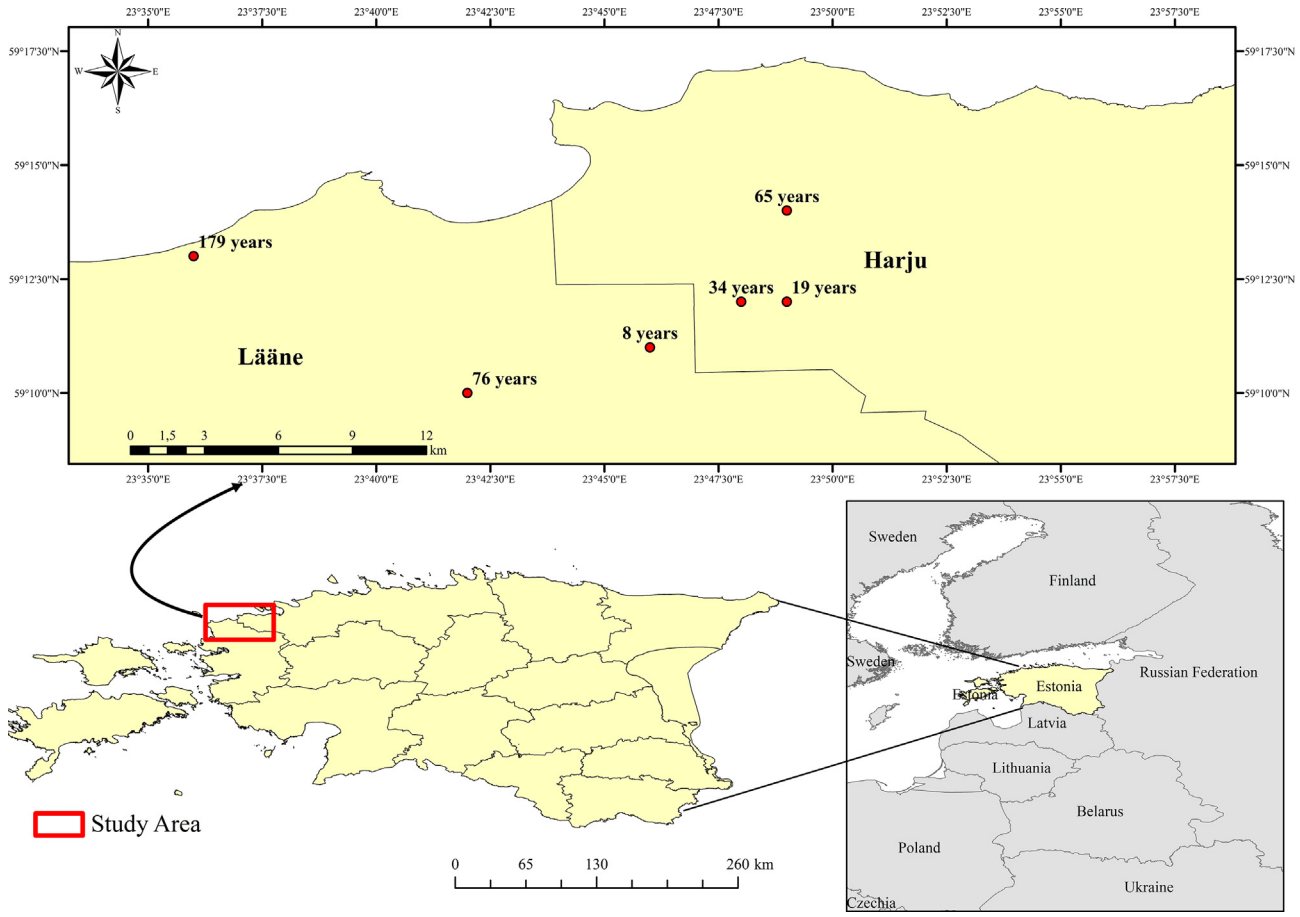

Fig. 1. Study area and age classes of the fire chronosequence in northwestern Estonia. Red dots represent the locations of age classes of successional stages following stand-replacing fires. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ple plot replicates that were randomly located at least 100 m apart from each other. Soils on the stands were classified as gleyic podzol (IUSS Working Group WRB, 2015) with loamy sand and belonged to the *Calluna* and *Vaccinium uliginosum* site type (Jõgiste et al., 2018; Lõhmus et al., 2004). The average annual temperature in the region is about 5.2 °C, and Scots pine (*Pinus sylvestris* L.) was the predominant tree species. Hereafter, we also refer to successional stage as time after fire and consider the last age class a pre-fire condition. The study areas had undergone no or minimal management actions prior to our measurements. The study areas comprising this chronosequence have been previously used to assess CO_2 effluxes and are described in more detail in Köster et al., 2016.

2.2. Flux chamber based measurements of CO_2, CH_4 and N_2O

We measured soil GHG fluxes once a month between May and October 2016 on 36 randomly located permanent collars along the fire chronosequence. The collars (22 cm diameter and 5 cm height) were placed at least 5 m apart from each other at 1–2 cm depth in the soil and sealed with sand to prevent gas leakage from the chamber to the atmosphere. Gas fluxes were measured during the daylight using a portable chamber (22 cm diameter and 24 cm height) made of Plexiglas and covered with a non-transparent plastic as in Pumpanen et al. (2015). The chamber had a built-in fan to maintain constant and homogenously increasing effluxes. The relative humidity and temperature inside the chamber were logged with an RH-/T-sensor (HMP75, Vaisala Oyj, Finland) for all GHG measurements.

A non-dispersive infrared CO_2 probe (GMP343, Vaisala Oyj, Finland) recorded CO_2 concentration inside the chamber at 5 s intervals for 5 min. The first 30 s from each measurement was discarded to reduce disruption. The CO_2 fluxes were calculated based on the change in gas concentration inside the chamber headspace, as previously described in Köster et al., 2016. The CH_4 and N_2O fluxes were sampled with a 50 mL polypropylene syringe (BD Plastipak 60, BOC Ohmeda, Sweden) connected to the same chamber. Gas fluxes were extracted just before chamber deployment (at 0 min) and at 1, 5, 10 and 20 min after chamber deployment. The gas was injected into 12 mL glass vials (Soda glass Labco Exetainer®, Labco Limited, UK) to be analysed by an Agilent Gas Chromatograph (7890A GC, Agilent Technologies, USA) equipped with a flame ionization detector (FID) and an autosampler (Verity GX-271 Liquid Handler, Gilson, USA). Helium (45 mL min^{-1}) was the carrier gas, synthetic air (450 mL min^{-1}) and hydrogen (40 mL min^{-1}) were flame gases, and nitrogen dioxide (5 mL min^{-1}) was the make-up gas of the FID. Detector and oven temperatures were set to 300 and 60 °C, respectively. We used the linear flux calculation method (Pihlatie et al., 2013) to estimate CO_2, CH_4 and N_2O chamber fluxes, and data points that deviated from the linear regression slope were filtered out as outliers.

2.3. Vegetation biomass and soil physicochemical attributes

To estimate below- and above-ground vegetation biomass, we measured basic tree characteristics (diameter, height, crown length, crown diameter, number of trees per hectare) from all three sample plots (radius 11.28 m (400 m^2)) located randomly on all fire

chronosequence areas. Tree biomass calculations were done according to Repola (2009). All dead wood longer than 1.3 m and at least 10 cm in diameter was included as dead-wood biomass. We sampled and dried at 60 °C ground vegetation samples from two squares (20 cm × 20 cm) per sample plot and categorized them as vascular plants (shrubs and grasses) and non-vascular organisms (mosses and lichens). Results are reported in kg dry weight of biomass m^{-2}. We collected five soil cores (50 cm long and 5 cm in diameter) from each sample plot for pH and total soil C and N analysis. Prior to the analysis, the different horizons were separated in organic and mineral layers, air-dried and then sieved through a 2 mm mesh. Soil pH was analysed with a glass electrode (Standard pH meter, Radiometer Analytical, Lyon, France) in 35 mL soil suspensions, at a 1:2.5 ratio of soil to ultrapure Milli-Q water, left overnight to stand after mixing. Next, the soil C and N content were determined by an elemental analyser (varioMAX CN Elementaranalysator, Elementar Analysensysteme GmbH, Germany) after samples had been oven dried at 105 °C for 24 h and grinded with a ball mill (Retsch, Germany). Soil temperature and soil moisture were measured during the flux measurements 10–20 cm from the collars at 5 cm depth with a digital thermometer (P 300w temperature probe, Dostmann Electronic GmbH, Germany) and with a soil moisture sensor (Thetaprobe ML3, Delta-T Devices Ltd, UK) connected to a data reader (HH2 moisture meter, Delta-T Devices Ltd, UK), respectively.

2.4. Litter-bag decomposition and extracellular enzyme activities

A litter-bag decomposition experiment (12 litter bags per age class) was conducted to determine changes in mass loss and moisture content of the litter bags, as well as the potential extracellular activity of seven indicator enzymes: cellobiohydrolase (CEL, EC 3.2.1.91), β-glucosidase (GLS, EC 3.2.1.21), β-glucuronidase (GLR, EC 3.2.1.31), β-xylosidase (XYL, EC 3.2.1.37), laccase (LAC, 1.10.3.2), N-acetylglucosaminidase (NAG, EC 3.2.1.14), and acid phosphatase (PHO, EC 3.1.3.2). CEL hydrolyses cellulose, GLS hydrolyses cellobiose into glucose, GLR is a lysosomal glycosidase that can detach carbohydrates from proteins, XYL degrades the xylose component of hemicellulose, LAC participates in the oxidation of lignin (Pollegioni et al., 2015), NAG degrades chitin, and PHO mineralizes organic phosphorus (P) into phosphatase.

Each litter bag, sized 7 cm × 10 cm and made of 0.2 mm nylon mesh, contained 5 g of air-dried Scots pine needles collected from Hyytiälä, Finland (61°51′N 24°17′E) in early spring of year 2015. The litter bags were buried between the litter and the humus layer in late spring, and left incubating for 1 year. Litter bags were replicated four times per sample plot, resulting in 72 litter bags altogether. After 1 year of decomposition, the litter bags were harvested and kept frozen at −21 °C.

Approximately 0.3 g of thawed litter from each litter bag was transferred into a 1.5 mL microcentrifuge tube. One hundred microlitres of sterile distilled water was added to the litter and left incubating at room temperature for 1 h. After incubation, the samples were centrifuged for 30 min. The resulting supernatant fluid was pooled and diluted with distilled water to a volume of 3 mL (Heinonsalo et al., 2012). The CEL, GLR, GLS, NAG, XYL and PHO assays used 4-methylumbelliferone substrates and the resulting fluorescence was measured using a Victor 1420 multi-label plate counter (PerkinElmer, Waltham, MA, USA) with an excitation wavelength of 460 nm. Activities of LAC were based on the enzymatic reaction of the sample with diammonium 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate, and the colorimetric reaction was quantified by a microplate spectrophotometer (Labsystems iEMS Reader MF *v*.2.9–0, Labsystems Oy, Finland) at 405 nm (Pritsch et al., 2011). Results are reported in nmol (enzyme substrate) g^{-1} dry weight of needles min^{-1} The remaining biomass in the litter bags was oven dried at 50 °C until a constant mass was reached, so mass loss and moisture content could be determined from the difference between the initial and the final dry mass of the litter.

2.5. Statistical analysis

The following statistical analyses were performed in R 3.5.1 (R Core Team, 2014) with specified packages. Data exploration was performed according to the protocol described in Zuur et al. (2010). To test our first hypothesis, the effects of successional stage, soil temperature, soil moisture, and interactions among them on GHG fluxes were explored using linear mixed models with restricted maximum likelihood estimations. The experimental design comprised repeated observations from multiple plots for each successional stage. Each collar was nested in a plot to incorporate the dependency among observations from the same collar within the same plot, ensuring a maximal random effects structure justified by the design (Barr et al., 2013). Fixed covariates were successional stage (categorical with six levels), soil temperature (continuous), and soil moisture (continuous). To assess whether the GHG soil emissions responded differently to soil temperature and soil moisture across the successional stages, we tested the significance of the following interaction terms: successional stage × soil temperature and successional stage × soil moisture. The full model form was:

$$Y_{ij} = \beta_0 + \beta_1 x_{ij1} + \beta_2 x_{ij2} + \beta_3 x_{ij1} x_{ij2} + \beta_4 x_{ij3} + \beta_5 x_{ij1} x_{ij3} + \gamma_{i0} + \gamma_{i1} + \varepsilon_{ij}\gamma_{i0} \sim N(0, \sigma^2_{\gamma_0}),\ \gamma_{i1} \sim N(0, \sigma^2_{\gamma_1}),\ \varepsilon_{ij} \sim N(0, \sigma^2_{\varepsilon})$$

where Y_{ij} is the *i*-th observed GHG flux (μg CH_4 or N_2O m^{-2} s^{-1}) of successional stage *j*, x_1 is successional stage (years) with index *j*, x_2 is soil temperature (°C) and x_3 is soil moisture (%). β represents the fixed parameters, γ represents the random intercepts associated with plot and collar, and ε is the error term.

For CO_2, a random intercept and slope model was fitted to account for the fluctuation in the relationship between CO_2 flux and soil temperature between months (Makita et al., 2018; Widén and Majdi, 2001):

$$Y_{ij} = \beta_0 + \beta_1 x_{ij1} + \beta_2 x_{ij2} + \beta_3 x_{ij1} x_{ij2} + \beta_4 x_{ij3} + \beta_5 x_{ij1} x_{ij3} + \gamma_{i0} + \gamma_{i1} + \gamma_{i2} x_{ij2} + \gamma_{i3} x_{ij2} + \varepsilon_{ij}\gamma_{i0} \sim N(0, \sigma^2_{\gamma_0}),\ \gamma_{i1} \sim N(0, \sigma^2_{\gamma_1}),\ \gamma_{i2} \sim N(0, \sigma^2_{\gamma_2}),\ \gamma_{i3} \sim N(0, \sigma^2_{\gamma_3}),\ \varepsilon_{ij} \sim N(0, \sigma^2_{\varepsilon})$$

where Y_{ij} is the *i*-th observed CO_2 flux (mg CO_2 m^{-2} s_{-1}) of successional stage *j*, x_1 is successional stage (years) with index *j*, x_2 is soil temperature (°C) and x_3 is soil moisture (%). β represents the fixed parameters, γ represents the random intercepts and slopes associated with plot, collar and month, and ε is the error term.

Full models represent the hypothesis that the interaction terms are significantly different from zero, while null models represent the hypothesis that the interaction terms are equal to zero. Models were fitted with the *lme4* package (Bates et al., 2015), and significance of interaction terms and individual covariates was tested using likelihood ratio tests with the *drop1* function. Marginal and conditional R^2 were calculated according to Nakagawa and Schielzeth (2013) with the *MuMIn* package (Bartón, 2018). Model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the model, and versus each covariate not in the model (Zuur et al., 2013). The differences in the least-square means of each GHG along the chronosequence were tested with *emmeans* (Lenth, 2018) using null models.

We also used linear mixed models, with plots as random effects, to test for a relationship between litter mass loss and moisture content, and to examine changes in litter mass loss, moisture content, and enzyme activities across successional stages. Activities of CEL, GLS, GLR, XYL and LAC were averaged and log-transformed to improve homogeneity of variance, so we could estimate the overall response of C-targeting enzymes. To determine the relationship

between enzyme activities and successional stages, we first categorized the activity rates as low, normal, and high based on the activity classes of the 30th and 70th percentiles, as suggested in Burns and Dick (2002), using the *dplyr* package (Wickham et al., 2018). Then, we verified the significance of successional stage for each enzyme activity using *drop1* on cumulative link models fitted using the *ordinal* package (Christensen, 2018).

A correlation-based principal component analysis (PCA) was performed in the JMP statistical software (JMP v. 14, SAS Institute Inc., Cary, NC, USA) to study the inter-association of the measured variables (GHG fluxes, average enzyme activities, vegetation biomass, soil temperature, soil moisture, soil pH, soil C and N content, depth of humus layer, mass loss, and moisture content of litter bags). Successional stages were projected onto principal component axes as supplementary variables to enrich the interpretation of the chronosequence scenarios. The association of each variable with a principal component was arbitrarily defined as strong (loading 0.67–1.0), moderate (loading 0.33–0.66), or weak (0.0–0.32). Loadings were considered as eigenvectors normalized to respective eigenvalues, and variables were scaled to unit variance. The relationships between environmental variables and GHGs were further assessed based on Pearson correlation coefficients ($-1 < \rho < 1$). All statistical analyses were performed with a significance level of 0.05.

3. Results

3.1. Fire effects on CO_2, CH_4 and N_2O fluxes

There was a distinct seasonal variation of soil respiration with a gradual emission increase towards summer and later decrease towards autumn in all successional stages except 8 years after fire (Fig. 2a). Mean soil respiration 8 years after fire (0.086 mg CO_2 - m^{-2} s^{-1}) was comparable with 19 and 34 years but was significantly lower than the latter successional stages (Fig. 2b). Mean CO_2 effluxes were 56% lower 8 years after fire compared with pre-fire levels and became almost threefold higher 76 years after fire. Soil was a sink (net uptake) for CH_4 in every successional stage, with highest mean uptake 8 years after fire (0.015 μg CH_4 - m^{-2} s^{-1}) and lowest 179 years after fire (0.008 μg CH_4 m^{-2} s^{-1}), but the model failed to detect significant differences between successional stages (Fig. 2c–d). Soil was both a sink and source of N_2O, but the direction of the fluxes was only significant between months ($P < 0.05$), with highest uptake in May (0.0017 μg N_2O m^{-2} s^{-1}) and highest emission in August (0.0012 μg N_2O m^{-2} s^{-1}) (Fig. 2e-f).

3.2. Soil greenhouse gas emissions in relation to plant biomass and soil physicochemical attributes

There was a gradual increase in mean soil temperature, peaking during the period from June to August, and a subsequent decrease in the following months (Table A.1). During the warmest months, mean soil temperature 8 years after fire was higher than pre-fire, approaching a 6 °C difference in June. Mean soil temperature over the entire measurement period was significantly higher 8 years after fire than 19, 34 and 65 years after. Soil moisture varied considerably during the measurement period. The differences seemed larger within successional stages from May until July. Soil moisture 8 years after fire increased in the course of the measurement period, being the lowest in May and highest in October, whereas pre-fire levels of moisture remained the same ($P > 0.05$). Mean soil moistures 19, 65 and 76 years after fire were the highest.

Soil respiration responded differently to both soil temperature and moisture across the successional stages (Table 1). The interactions between successional stage and soil temperature, and

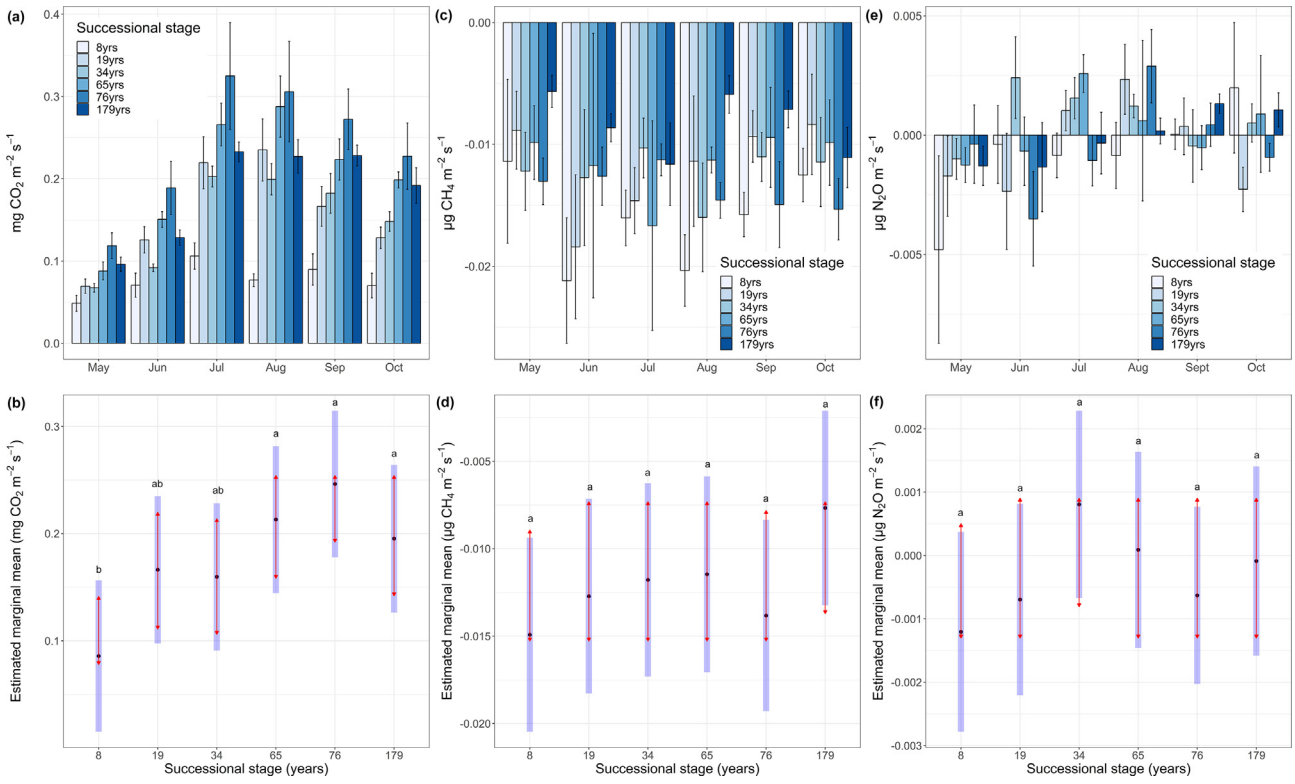

Fig. 2. a–c–e Gas fluxes based on chamber measurements conducted in 2016. Bars show means, and error bars show standard errors (n = 6). b–d–f Estimated mean difference for each respective gas between successional stages derived from null models fitted by restricted maximum likelihood. Black dots mark the mean, and bars are 95% confidence intervals for the means. Different letters and non-overlapping arrows from different successional stages indicate a significant difference ($P < 0.05$).

Table 1
Effects of successional stage, soil temperature, soil moisture, and their interactions on CO_2, CH_4 and N_2O using linear mixed models based on a fire chronosequence *in situ* experiment conducted in 2016. Likelihood ratio tests (χ^2), degrees of freedom (df), and *P*-values (*P*) were calculated by applying the *drop1* function on full and null mixed models fitted with maximum likelihood (ML). Significant covariates ($P < 0.05$) are marked in bold.

Covariates	CO_2			CH_4			N_2O		
	$\chi2$	df	*P*	$\chi2$	df	*P*	$\chi2$	df	*P*
Successional stage	20.98	5	**<0.001**	5.88	5	0.318	5.46	5	0.362
Soil temperature	0.32	1	0.572	4.43	1	**<0.05**	5.36	1	**<0.05**
Soil moisture	0.46	1	0.498	0.21	1	0.650	0.98	1	0.322
Successional stage × Soil temperature	25.49	5	**<0.001**	4.62	5	0.465	3.44	5	0.633
Successional stage × Soil moisture	18.91	5	**<0.05**	1.82	5	0.874	6.93	5	0.226

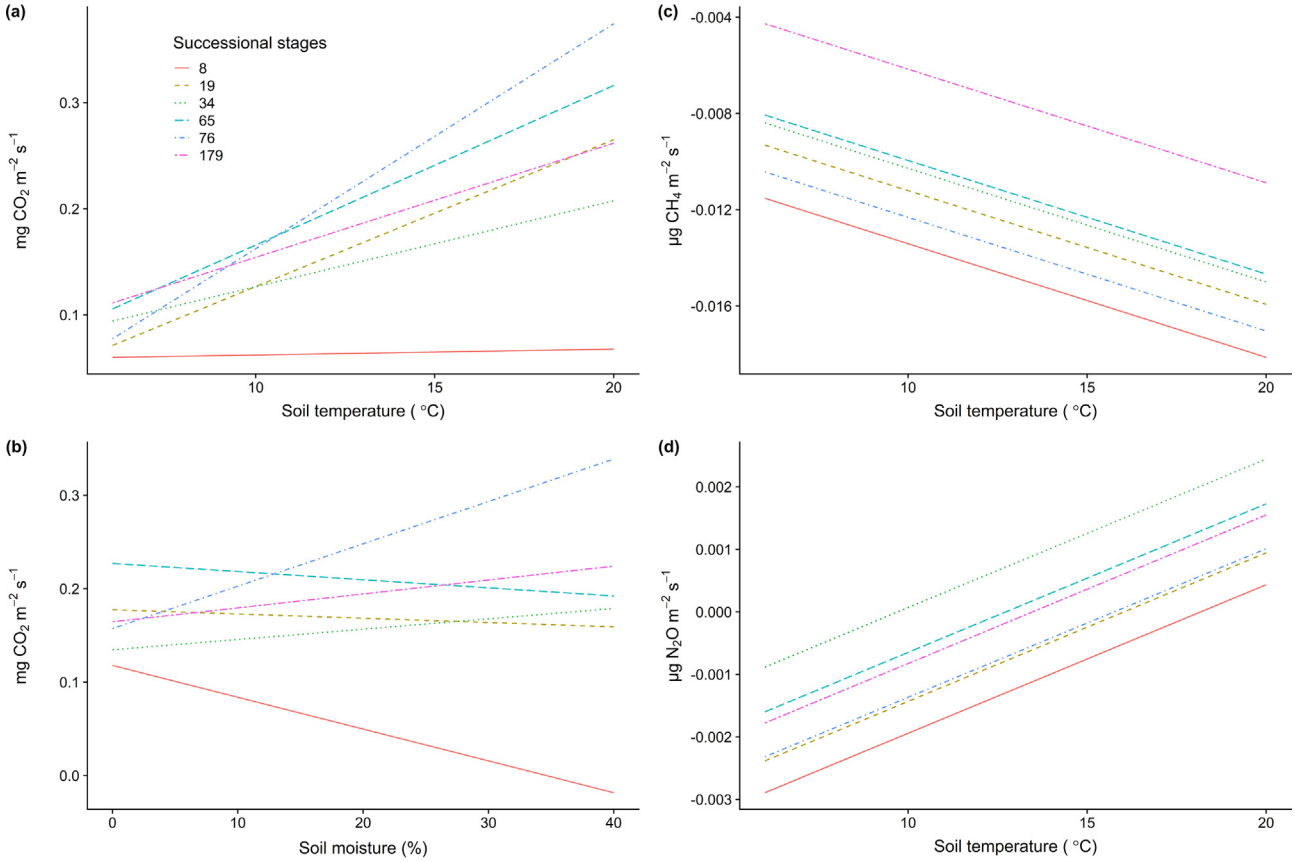

Fig. 3. Linear mixed model predictions showing significant relationships between greenhouse gas fluxes and measured soil parameters for each successional stage. a CO_2 and soil temperature (soil moisture was held constant). b CO_2 and soil moisture (soil temperature was held constant). c CH_4 and soil temperature (soil moisture was held constant). d N_2O and soil temperature (soil moisture was held constant). The predicted relationships for CO_2 are based on full models, and the relationships for CH_4 and N_2O are based on null models available in the supplementary material, Table A.2.

between successional stage and soil moisture, explained 41% of the variance (Table A.2). The effect of soil temperature on CO_2 emission was significantly higher 19, 65, 76 and 179 years after fire compared with 8 years (Fig. 3a; Table A.2). Soil respiration decreased with soil moisture 8 years after fire, but this relationship only significantly differed from 34 and 76 years after fire, when soil respiration increased with moisture (Fig. 3b; Table A.2). To verify whether the apparent negative effect of soil moisture on soil respiration 8 years after fire was related to the weak effect of soil temperature on respiration, we examined the significance of the relationship between soil temperature and soil moisture 8 years after fire. We did not detect a significant effect of soil temperature on soil moisture ($P = 0.1355$).

The response of both CH_4 and N_2O fluxes to soil temperature and moisture was indistinguishable between successional stages (Table 1). Soil temperature was the sole important factor for predicting both CH_4 uptake and N_2O emissions among the measured parameters (Table 1). Soil temperature positively affected CH_4 uptake and N_2O emission across successional stages similarly (Fig. 3c-d; Table A.2).

Above- and below-ground biomass changed drastically after fire disturbance (Table 2). Biomass of living tree stems and branches and needles were significantly lower in the first two decades of the succession, increasing tenfold 34 years after fire (2.25 ± 0.46 k g m^{-2}, $P < 0.05$). Living root biomass also increased during the succession, becoming significantly higher 34 years after fire than after the first 8 years (Table 2). Meanwhile, dead root biomass decreased considerably during the succession, ranging from 0.89 ± 0.40 kg m^{-2} in the initial 8 years to 0.19 ± 0.03 kg m^{-2} ($P < 0.01$) 19 years after fire. Differences in mean biomass of vascular plants were greater between 19 and 34 years after fire, whereas no significant changes were detected in moss (and lichen) biomass. In addition to soil

Table 2
Mean above- and below-ground biomass (kg m^{-2}) in the studied fire chronosequence. Values represent least-square means (standard deviation) for study areas (age classes). Different subscript letters indicate significant differences between the fire age classes (n = 3) at $P < 0.05$. Pearson's correlation coefficients (ρ) are provided to verify the association between measured greenhouse gases (CO_2, CH_4, N_2O) and vegetation biomass. Significant correlations are marked in bold ($P < 0.05$).

Age classes (years)	Living roots	Dead roots	Coarse woody debris (CWD)	Tree stems	Vascular plants	Mosses and lichens	Branches and needles
8	0.39 $(0.20)_c$	0.89 $(0.40)_a$	3.02 $(1.60)_a$	0.04 $(0.04)_d$	0.23 $(0.08)_{bc}$	0.19 $(0.15)_a$	0.06 $(0.05)_c$
19	0.77 $(0.04)_c$	0.52 $(0.06)_{ab}$	1.41 $(0.21)_{ab}$	0.43 $(0.23)_d$	0.63 $(0.04)_a$	0.08 $(0.06)_a$	0.43 $(0.12)_c$
34	2.34 $(0.31)_b$	0.03 $(0.01)_c$	0.09 $(0.04)_b$	2.56 $(0.56)_c$	0.02 $(0.03)_c$	0.34 $(0.26)_a$	1.84 $(0.23)_b$
65	3.78 $(0.53)_a$	0.14 $(0.12)_{bc}$	0.39 $(0.36)_b$	8.41 $(1.55)_a$	0.43 $(0.25)_{ab}$	0.16 $(0.13)_a$	3.43 $(0.59)_a$
76	3.04 $(0.51)_{ab}$	0.11 $(0.08)_{bc}$	0.33 $(0.24)_b$	6.07 $(0.83)_b$	0.22 $(0.08)_{bc}$	0.11 $(0.06)_a$	3.07 $(0.14)_a$
179	4.18 $(0.60)_a$	0.19 $(0.04)_{bc}$	0.40 $(0.22)_b$	5.98 $(0.32)_b$	0.21 $(0.11)_{bc}$	0.30 $(0.06)_a$	2.92 $(0.14)_a$
ρ (CO_2)	**0.667**	**−0.669**	**−0.667**	**0.692**	0.229	−0.079	**0.711**
ρ (CH_4)	**0.288**	**−0.420**	**−0.450**	0.151	−0.145	−0.235	0.226
ρ (N_2O)	0.146	**−0.481**	**−0.480**	0.010	**−0.262**	**0.415**	0.111

Table 3
Mean soil thickness of the humus layer (n = 15), soil pH (n = 15), total soil carbon and nitrogen, and C:N ratios (n = 15) in the studied fire chronosequence. Values represent least-square means (standard deviation) for study areas (age classes). Different subscript letters indicate significant differences between the fire age classes. Pearson's correlation coefficients (ρ) are provided to verify the association between measured greenhouse gas (CO_2, CH_4, N_2O) and environmental variables. Significant correlations are marked in bold ($P < 0.05$).

Age classes (years)	Thickness (cm)	pH	C content (kg m^{-2})	N content (g m^{-2})	C:N ratio
8	2.1 $(1.1)_b$	3.99 (0.26)	0.53 $(0.4)_{bc}$	25.33 $(11.7)_b$	24.1
19	10.8 $(3.9)_a$	4.00 (0.36)	3.90 $(2.9)_a$	295.26 $(176.4)_a$	15.5
34	1.6 $(0.9)_b$	3.96 (0.17)	0.67 $(1.0)_b$	22.91 $(32.5)_b$	39.2
65	5.2 $(0.6)_b$	3.82 (0.16)	3.42 $(2.2)_{ac}$	122.91 $(80.2)_b$	28.2
76	3.0 $(2.8)_b$	3.64 (0.25)	1.75 $(0.8)_{ab}$	53.93 $(24.5)_b$	32.4
179	3.3 $(0.9)_b$	3.71 (0.09)	1.10 $(0.5)_{bc}$	27.52 $(10.9)_b$	39.7
ρ (CO_2)	**0.265**	−0.130	**0.405**	0.197	0.154
ρ (CH_4)	0.145	0.101	0.132	0.132	0.054
ρ (N_2O)	−0.002	**0.276**	0.060	−0.015	0.046

temperature and moisture, other environmental factors differed between successional stages (Table 3). Mean soil pH at the eluvial horizon was slightly higher at the beginning of the succession, reaching the lower point 76 years after fire (Table A.3). Soil pH of the humus and other mineral layers was slightly higher in the first age classes, but the differences were not significant.

Soil C and N pools of the organic and mineral horizons 8 years after fire were comparable with 179 years after fire, although they were significantly higher 19 years after fire. Concurrently, the thickest humus layer was found 19 years after fire, whereas no significant differences were verified between the other years (Table 3).

Further analysis showed that the measured GHGs strongly correlated with vegetation biomass (Table 2) and weakly to moderately with soil physicochemical properties (Table 3). All gases correlated negatively with coarse woody debris (CWD) and with dead root biomass. Negative significant correlations were also found between N_2O fluxes and biomass of vascular plants. We found positive significant relationships between CO_2 efflux and branches and needles, tree stems, and living roots; between CH_4 uptake and living roots; and between N_2O fluxes and moss and lichen biomass. Moreover, CO_2 efflux significantly correlated with thickness and the C content of the humus horizon, whereas N_2O was the only GHG that significantly correlated with pH of the humus horizon. We did not detect significant relationships between CH_4 uptake and measured environmental factors other than soil temperature.

3.3. Litter mass loss, moisture content, and potential extracellular enzyme activities

Litter bags decomposing in the pre-fire study area lost on average 15% more mass than litter decomposing 8 years after fire (Fig. 4a), indicating higher enzymatic activity in older stands. The moisture content of litter bags was statistically the same in the first three successional stages but almost doubled in the latter successional stages. It also explained 20% of the variation in needle mass loss (Fig. 4b).

The average potential extracellular activity of all measured C-targeting enzymes was similar across successional stages ($P = 0.119$; Fig. 5). Fire significantly reduced potential extracellular enzyme activities of XYL, CEL, NAG and PHO ($P < 0.05$; Fig. 6; Table A.4), with a lasting reduction in activity of at least 8 years.

3.4. Fire chronosequence scenarios

The PCA produced five components with an eigenvalue above 1.0, which explained 83.4% of the total variance (Table A.5) among the 18 original variables. PC1 and PC2 explained 28% and 25.7% of the total variance, respectively. Among the strongest correlations to PC1, litter mass loss and CO_2 fluxes correlated positively, whereas biomass of dead roots, total CWD, and soil temperature correlated negatively (Fig. 7; Table A.6). Among the strongest correlations to PC2, N content of the humus horizon, C content of the humus and mineral horizons, depth of the humus horizon, and soil moisture correlated positively, whereas only the C:N ratio of the humus horizon negatively correlated to PC2. In turn, 8 years after fire was better represented by the negative PC1 axis, 19 years after fire by the positive PC2 axis, while 179 years after fire was better represented by the negative PC2 axis (Fig. 7; Table A.7). The remaining successional stages were weakly represented by the components.

4. Discussion

The results showed that, under the conditions of our study areas (hemiboreal Scots pine forests), fire significantly reduced the soil CO_2 efflux, while it had no effect on CH_4 or N_2O fluxes. There was a reduction of soil CO_2 efflux at the beginning of the fire chronosequence but no changes in CH_4 or N_2O fluxes related to time after fire. Fire had minor effects on potential extracellular

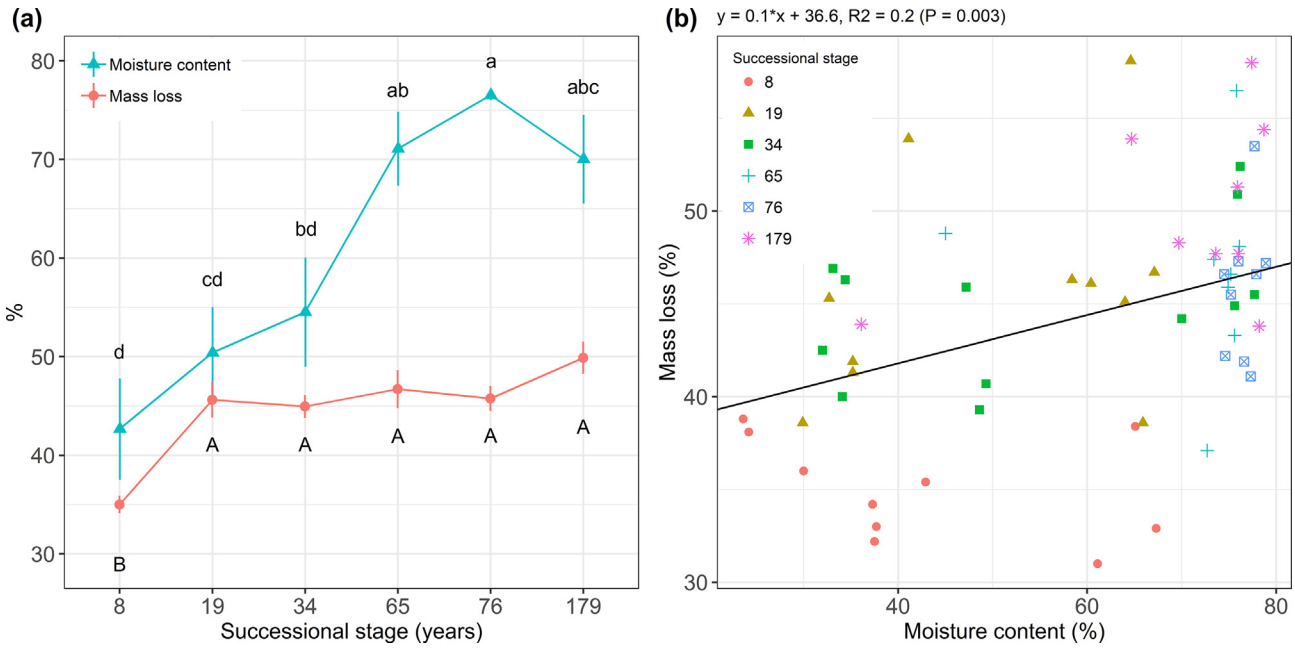

Fig. 4. a Average needle mass losses (%) and litter moisture content (% of fresh weight) for litter bags (Scots pine needles). Points are mean and error bars are standard errors (n = 12). Different uppercase letters below bars denote significant differences ($P < 0.05$) in needle mass loss between successional stages. Different lowercase letters above bars denote significant differences ($P < 0.05$) in litter moisture content between successional stages. b Relationship between litter mass loss and moisture content of litter bags.

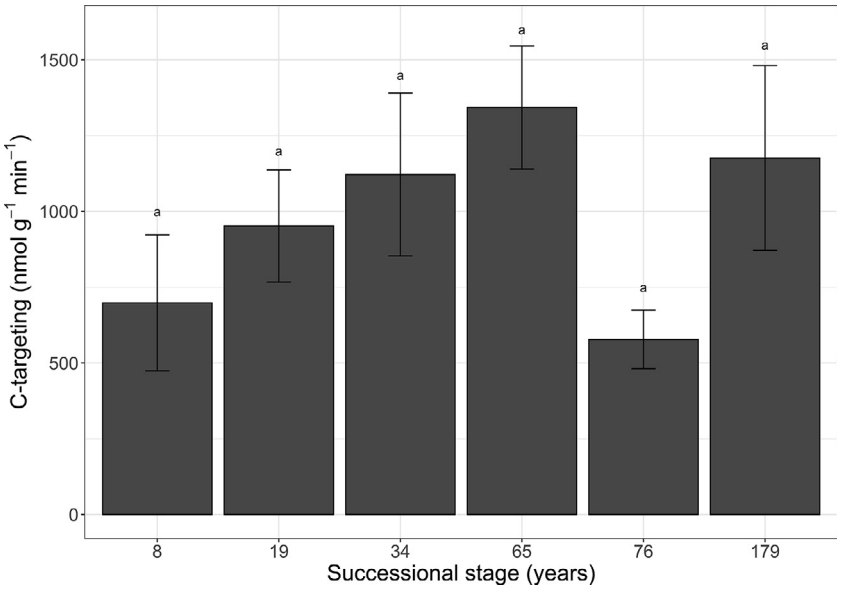

Fig. 5. Average of C-targeting enzymes (cellobiohydrolase, β-glucosidase, β-glucuronidase, β-xylosidase, laccase) from decomposing needle litter (nmol g^{-1} dry weight of needles min^{-1}) across successional stages (n = 12). Error bars are standard errors. Different letters above bars denote statistically significant differences ($P < 0.05$).

enzyme activities from decomposing needle litter, and these persisted for less than 19 years.

4.1. Fire effects on soil CO_2, CH_4 and N_2O fluxes

We found that soil respiration 8 years after fire significantly differed from pre-fire levels, with the mean CO_2 efflux increasing across the post-fire succession. These results are in accordance with our earlier observations in boreal forests, which showed that CO_2 effluxes increase with time after fire for both stand-replacing (Köster et al., 2017a; Köster et al., 2018) and non-replacing fires (Köster et al., 2014, 2015b). We found a CH_4 sink throughout the chronosequence, and the mean uptake tended to decrease with time after fire, but no significant difference between age classes was noted. These results suggest that environmental factors, such as soil temperature and moisture, driving CH_4 fluxes are not always conditional on time after fire. Lack of response to time after fire has been reported before (Kim, 2013; Köster et al., 2018) but, most often, long-lasting effects of fire on CH_4 uptake populate the literature (Burke et al., 2004; Köster et al., 2015a; Köster et al., 2017a; Song et al., 2018). Short-term fire effects are more irregular, with CH_4 flux strength and direction varying greatly during the first months to a few years after fire (Jaatinen et al., 2004; Kulmala et al., 2014; Nakano, 2006). Consistent with the literature (Castro

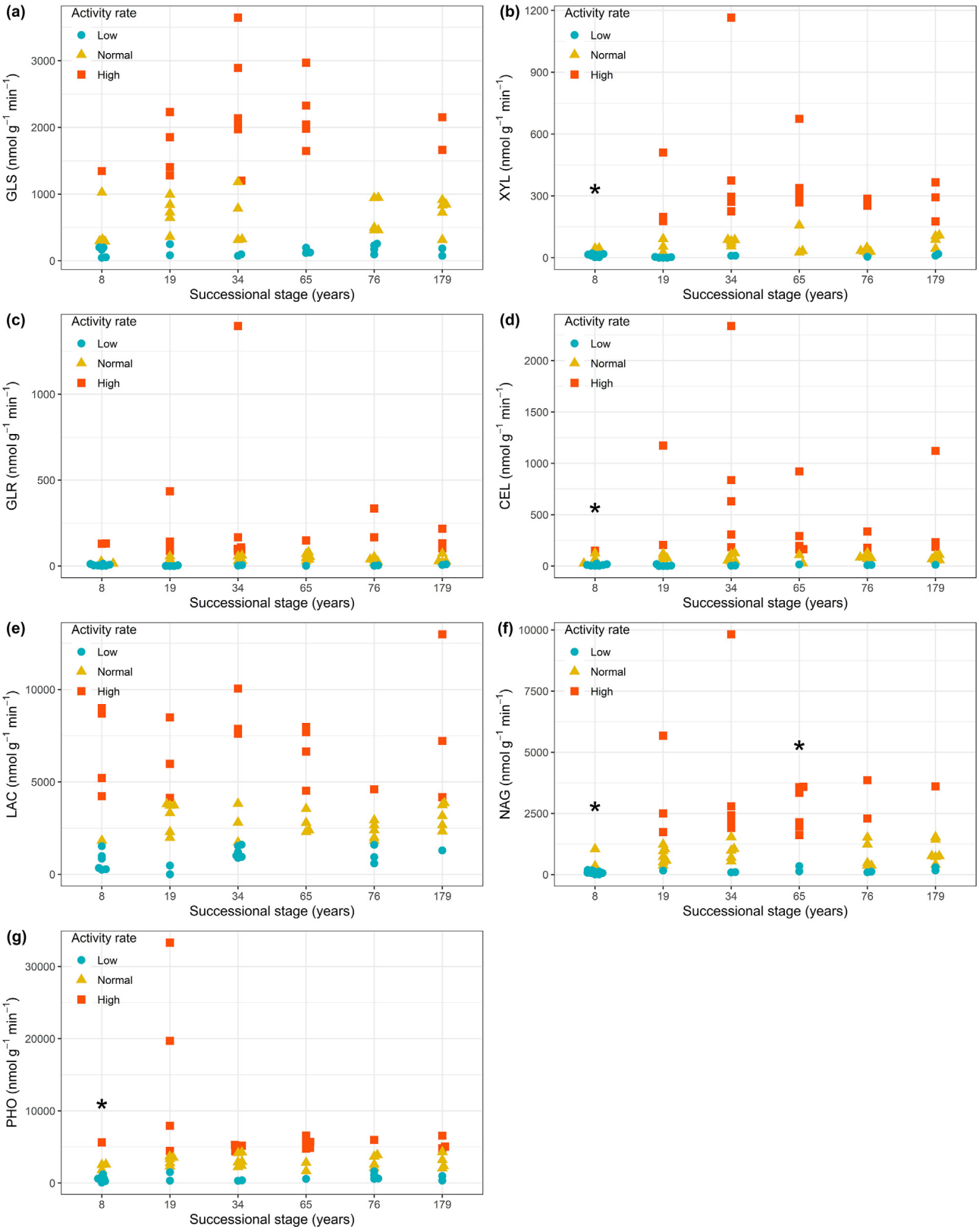

Fig. 6. Scatter plot of raw enzyme activity from decomposing needle litter (nmol g^{-1} dry weight of needles min^{-1}) categorized by rates (low, normal and high) across successional stages (n = 12). Asterisk (*) indicates the age classes that differed significantly ($P < 0.05$) from 179 years after fire in terms of enzyme activity.

et al., 1992; Klemedtsson et al., 1997), the studied soil acted as both a sink and source of N_2O, at least between months (sampling dates), with mean N_2O uptake more often occurring at the beginning of summer. In fact, the highest mean N_2O uptake occurred in May and the highest N_2O emission in August, when the mean soil temperature was the lowest and the highest, respectively. However, no significant differences were detected between successional stages nor was a temporal trend identified. This result

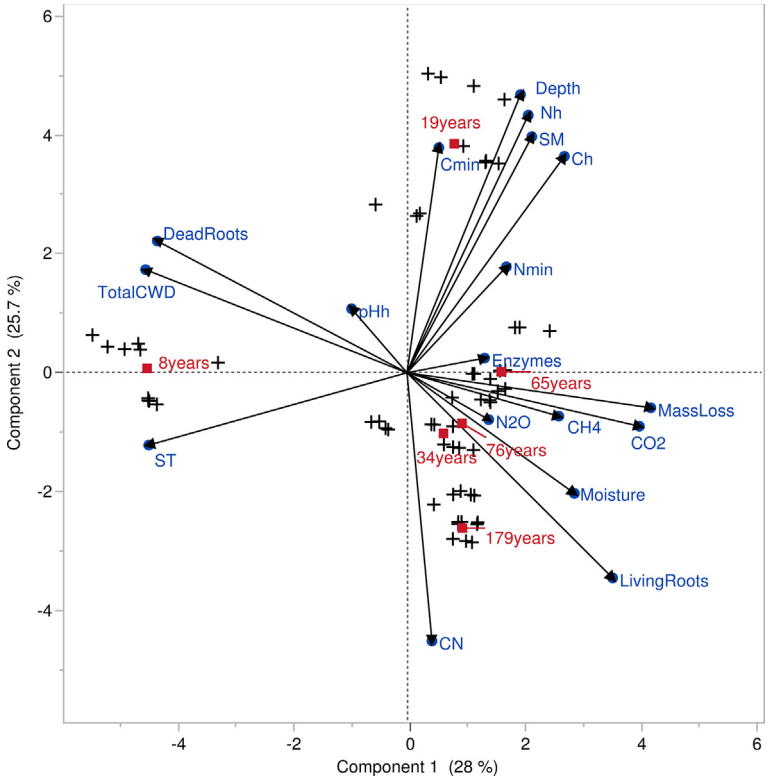

Fig. 7. Results of principal component analysis of the following variables: greenhouse gases (CO_2, N_2O, CH_4), living and dead root biomass (LivingRoots, DeadRoots), total coarse woody debris (TotalCWD), soil temperature (ST), soil moisture (SM), soil pH of the humus layer (pHh), soil C and N content of the humus and mineral layers (Ch, Cmin, Nh, Nmin), C:N ratio of humus layer (CN), depth of humus layer (Depth), mass loss and moisture content of litter bags (MassLoss, Moisture), and average of all measured enzyme activities (Enzymes). Plus signs represent component scores, arrows represent factor loadings for each principal component, and squares represent successional stages.

reflects that from boreal forests of Köster et al. (2015a) who also found no differences in N_2O fluxes during a long-term fire chronosequence.

4.2. Soil greenhouse gas emissions in relation to plant biomass and soil physicochemical characteristics

The mixed models predicted different responses of CO_2 emissions to soil temperature and moisture across the successional stages but not the responses of CH_4 and N_2O fluxes. The model predicted a negative effect of soil moisture on CO_2 efflux 8 years after fire, whereas 34 and 76 years after fire, moisture had a positive effect on CO_2 efflux. The stands aged 19, 65 and 76 years had the highest moisture levels, but moisture content varied considerably both within measurement period and across successional stages. As we did not detect a significant effect of soil temperature on soil moisture, a different factor might explain the negative effect of soil moisture on CO_2 efflux at the beginning of the fire succession. After fire, ash and clay minerals may clog soil pores, reducing water infiltration and gas diffusion while bulk density increases (Certini, 2005). Post-fire increase in bulk density has been related to a decrease in soil respiration (Saiz et al., 2006). Supposing that fire increased bulk density up to 8 years after fire, gas diffusion could become more sensitive to moisture fluctuation. As expected, the effect of soil temperature on CO_2 emissions also changed during the fire chronosequence. It was significantly weaker 8 years after fire compared with 19, 65, 76 and 179 years after fire, despite soil temperature being the highest during the same time. It is interesting to note that in the PCA, both CO_2 fluxes and litter mass loss were negatively associated with soil temperature, dead root biomass, and CWD, which were the most important variables 8 years after fire. Comparison of these findings with those of other studies (Holden et al., 2015; Treseder et al., 2004) confirms that the loss of plant root and microbial and fungal biomass at the beginning of the fire succession cannot be entirely compensated by an increase in soil temperature.

The largest seasonal variation of soil temperature occurred 8 years after fire, which was also the area with the lowest average plant biomass. The post-fire increase in soil temperature has been previously attributed to the combustion of vegetation and the humus layer (Liu et al., 2005; Sullivan et al., 2011; Treseder et al., 2004), which besides directly increasig incoming solar radiation, has been observed to alter albedo for several decades (Amiro et al., 2006; Randerson et al,. 2006). In our study, soil temperature returned to pre-fire levels before above- and belowground vegetation showed significant growth. This indicates that the condition of the organic layer and ground vegetation was suitable to provide insulation before 34 years after fire. We must interpret these results with caution, as thickness of the humus layer and C and N stocks are strangely large 19 years after fire, suggesting that the fire severity in this study area was not as high as in the others. Although no longer present in our study areas, ash deposition has also been reported to provide insulation proportional to its layer depth, ultimately reducing extreme soil temperature (Bodí et al., 2014).

The stand-replacing fires drastically reduced above- and belowground plant biomass of the forest stands. Vegetation biomass – quantified as living tree stems, branches, needles, and living roots – increased significantly only 34 years after fire. However, CO_2 efflux had already stabilized at least 15 years before. This finding implies that up to 34 years after fire, a greater share of total soil respiration likely results from litter and SOM decomposition. Root

turnover and CWD decomposition, including charred plant materials, are critical for C recovery (González-Pérez et al., 2004; Homann et al., 2015; Singh et al., 2008), while the fire-related reduction of the humus layer and C stocks rank as one of the main reasons for the reduction in soil respiration (Bergner et al., 2004; Gupta and Mackenzie, 2016; O'Donnell et al., 2009; Sullivan et al., 2011). In support of this interpretation, dead coarse roots and CWD biomass were significantly higher at the beginning of the succession. Thus, despite the loss of living plant biomass, below-ground litter (i.e. organic matter from dead roots) may become eventually and temporarily greater than in the absence of forest fire (Köster et al., 2016). Moreover, according to the PCA, dead root biomass and CWD best characterized 8 years after fire areas, and C and N pools and depth of humus horizon characterized 19 years after fire areas. Additionally, we found a strong significant temporal correlation between CO_2 efflux and living root biomass and only a moderate temporal correlation between CO_2 efflux and total soil C. Because only microbial respiration represents loss of stored C (O'Neill et al., 2002), the combination of these results suggests that a large part of soil CO_2 production in older forest stands will likely be autotrophic. This finding is consistent with that of Goulden et al. (2011), who described a relatively similar CO_2 efflux trend to the one in this study, in which total respiration strongly mimicked variation in root respiration after a stand-replacing fire, while heterotrophic respiration remained steady throughout the chronosequence. Despite not finding significant relationships between soil respiration and estimated ground vegetation, vascular plants and mosses had already partially recovered prior to the first age class of the chronosequence. Therefore, even though the impairment of roots by fire during the first years of the succession may reduce total soil respiration (O'Neill et al., 2006), the quick recovery of ground vegetation and concomitant inputs of root exudates and litter may mitigate some of this loss (Burke et al., 2004; Köster et al., 2015a).

According to the mixed model, soil temperature was the factor that explained most of the variation in CH_4 fluxes. A positive effect of temperature on CH_4 uptake after fire in permafrost areas has been consistently reported (Morishita et al., 2015; Song et al., 2017, 2018; Takakai et al., 2008), but the evidence for this relationship is inconclusive in non-permafrost areas. Besides soil temperature, Köster et al. (2015a) suggested that fire-induced changes to soil C stocks and microbial biomass control CH_4 uptake. The current study has been unable to demonstrate the direct effect of organic horizon depth, C stocks, or microorganisms on CH_4 flux, as we did not detect significant relationships between these variables. Nevertheless, the low R^2 of the mixed model indicates that other factors explain CH_4 fluxes at our sites. Among all tested correlations, CH_4 fluxes were only associated with vegetation biomass – positively with living roots and negatively with dead roots and CWD. Thus, the decrease in mean CH_4 uptake seems to follow the increase in living root biomass. Methane emissions from tree stems have been recently discovered, although it is not yet known whether the source of CH_4 emitted from tree stems is the soil itself, transported to the surface by roots, or the heartwood of the tree (Barba et al., 2019). Overall, these results suggest that fire-induced higher soil temperature and re-establishment of the overstorey have competing effects on CH_4 fluxes. On one side, soil temperatures seem to contribute to CH_4 uptake, whereas subsequent growing trees might counterbalance some of this uptake by emitting CH_4.

The mixed model predicted higher temperatures to favour N_2O emissions and lower temperatures to favour N_2O uptake similarly in all age classes. N_2O emission is mainly driven by denitrification but can also occur by nitrification processes. The latter occur under aerobic conditions, whereas the former is favoured by anaerobic conditions (Ussiri and Lal, 2013). Soil temperature regulates both processes, being reported as a key driver of N_2O emission after fires (Kim and Tanaka, 2003; Köster et al., 2017a). Moreover, N_2O fluxes negatively correlated with biomass of vascular plants and positively with biomass of mosses and lichens, suggesting that N_2O emissions are favoured in forests where bryophyte and lichen species are more abundant in relation to vascular plants. The estimated contribution of known bryophyte and lichen species that are sources of N_2O corresponds to 4–9% of the total natural terrestrial N_2O emissions (Zhuang et al., 2012), and associated emissions likely occur during denitrification (Lenhart et al., 2015). Incidentally, these results may explain the negative observed correlation between N_2O fluxes and vascular plants, assuming that the relative contribution of mosses and lichens to N_2O emission is higher where vascular plants tend to be absent. The weakening of N_2O influx related to increased moss coverage after reindeer-grazing disturbance has also been recently described (Köster et al., 2017b). Another important finding was that N_2O fluxes positively correlated with soil pH. A pH increase has been associated with an increase in nitrification (De Boer and Kowalchuk, 2001), although acid-tolerant nitrifiers are also thought to exist in forest soils (Yao et al., 2011). In acid conditions, the enzyme responsible for N_2O uptake is inactive (Knowles, 1982). However, Chapuis-Lardy et al. (2007) note how the effect of soil pH on N_2O uptake is not straightforward. Taken together, these results suggest that N_2O emissions occur from both nitrification and denitrification processes. Because we did not measure nitrification and denitrification rates, it is difficult to precisely infer their occurrence temporally. It seems possible that during the beginning of the studied chronosequence, when mean soil temperature and pH were higher, nitrification contributed to N_2O emissions. Meanwhile, the input of moss and lichen through denitrification probably varied throughout the succession according to the abundance of species that are sources of N_2O.

4.3. Fire effects on enzyme activities, litter mass loss, and moisture of litter bags

The long-term fire effect on potential extracellular enzyme activities in decomposing needle litter was subtle. We could no longer detect a fire effect on the overall activity of C-targeting enzymes. Of the individual C-, N- and P-targeting enzymes in which we detected change in activity (XYL, CEL, NAG and PHO), the fire effect persisted for at least 8 years, but activity recovered by 19 years after fire. From the standpoint of a set of enzyme activities being an indicator of microbial activity (Burns and Dick, 2002), the enzyme assays signalled that fire effects on soil microbiology were somewhat short-lived in our study areas. As extracellular enzymes interact with clay minerals and humic molecules of the soil matrix, they are less sensitive to changes in environmental conditions (Burns, 1982; Dilly and Nannipieri, 2001). The recovery of extracellular enzyme activity has been previously observed to take from 7 to 60 years (Holden et al., 2013; Köster et al., 2015b), but in some cases fire might even increase enzyme activity (Taş et al., 2014). Nevertheless, the recovery of enzyme activity after fire has been associated with increased fungal biomass, CO_2 efflux, C stocks, and litter mass loss (Köster et al., 2015a). In our study, litter mass loss was the lowest 8 years after fire, but it reached pre-fire levels by 19 years after fire. A decrease in mass loss has been previously linked to a decrease in microbial-mediated litter decomposition (Holden et al., 2013). Given the importance of the recovery of decomposers to soil CO_2 efflux during the first years post-fire disturbance (Treseder et al., 2004), our results suggest that possible quick recovery of microbiota minimized adverse effects of fire on total soil respiration through impairment of root respiration during the first few decades of the post-fire succession.

Moisture content of the litter bags increased with time after fire and significantly correlated with most of the studied enzyme activities, stressing the dependency of the microbiota on soil moisture conditions. It also explained some of the variation in mass loss, but the fit for observations from 8 years after fire is especially bad, indicating a plausible null effect on CO_2 efflux. Thus, it appears that neither soil moisture nor moisture content of litter bags have a clear response in relation to mass loss or direct CO_2 efflux 8 years after fire. Connecting these results with the competing soil moisture effect on CO_2 efflux between the successional stages and with the inconsistency in soil moisture levels during the succession raises questions regarding the extent of the effect of moisture on soil respiration when sources of soil respiration are limited (e.g. C substrate, living roots and rhizosphere).

5. Conclusions

This study was undertaken to explore how fire-induced changes to soil properties and vegetation affect gas exchange of CO_2, CH_4 and N_2O in the soil–atmosphere interface of hemiboreal Scots pine forests in Estonia. We identified a reduction of soil CO_2 efflux at the beginning of the fire chronosequence, but no changes to CH_4 or N_2O fluxes related to time after fire. Confirming our first hypothesis, soil respiration responded differently to changes in soil temperature and soil moisture across the fire chronosequence. Conversely, CH_4 and N_2O fluxes only responded to changes in soil temperature. Recovery of soil respiration in the long term is associated with the moderate effect of fire on enzyme activity, the above- and below-ground litter C input, and the re-establishment of overstorey vegetation. Confirming our second hypothesis, enzyme activity and decomposition inside the litter bags were especially good indicators of the role of the microbiota on the initial recovery of soil respiration prior to the re-establishment of the vegetation. The generalizability of these results is subject to certain limitations that are likely to occur when employing a chronosequence approach. For instance, it was not possible to assess the effects of fire on soil physicochemical characteristics in relation to GHGs 19 years after fire, as the fire in the area could have been less severe than reported. Thus, soil respiration might have taken longer than 19 years to stabilize. Despite its limitations, the study certainly adds to our understanding of the importance and complexity of both above- and below-ground (vegetation and microbiota) responses to fire-induced changes of soil physicochemical properties for determining the temporal variability of gas exchange.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135291.

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