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Nitrogen and phosphorus enrichment effects on CO_2 and methane fluxes from an upland ecosystem

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ABSTRACT

Reactive nitrogen (N) deposition can affect many ecosystem processes, particularly in oligotrophic habitats, and is expected to affect soil C storage potential through increases in microbial decomposition rate as a consequence of greater N availability. Increased N availability may also result in changes in the principal limitations on ecosystem productivity. Phosphorus (P) limitation may constrain productivity in instances of high N deposition, yet ecosystem responses to P availability are poorly understood. This study investigated CO_2 and CH_4 flux responses to N and P enrichment using both short- (1 year) and long-term (16 year) nutrient addition experiments. We hypothesised that the addition of either N or P will increase CO_2 and CH_4 fluxes, since both plant production and microbial activity are likely to increase with alleviation from nutrient limitation. This study demonstrated the modification of C fluxes from N and P enrichment, with differing results subject to the duration of nutrient addition. On average, relative to control, the addition of N alone inhibited CO_2 flux in the short-term (-9%) but considerably increased CO_2 emissions in the long-term (+35%), reduced CH_4 uptake in the short term (-90%) and reduced CH_4 emission in the long term (-94%). Phosphorus addition increased CO_2 and CH_4 emission in the short term (+20% and +184% respectively), with diminishing effect into the long term, suggesting microbial communities at these sites are P limited. Whilst a full C exchange budget was not examined in the experiment, the potential for soil C storage loss with long-term nutrient enrichment is demonstrated and indicates that P addition, where P is a limiting factor, may have an adverse influence on upland soil C content.

Keywords:

Nitrogen deposition; soil carbon; carbon fluxes; pollution; co-limitation; P limitation

1.1 INTRODUCTION

Global climate change is expected to have profound impacts on natural systems, which could threaten biodiversity and ecosystem processes (Walther et al. 2002; Thomas et al. 2004; Grim et al. 2013; Carroll et al. 2015). In northern temperate ecosystems, carbon (C) has been accumulating in terrestrial reservoirs since the end of the last ice age, sequestered mainly as organic matter in soils with low rates of decomposition such as peat (Yu et al. 2010). Maintaining these C stores is important to avoid the transformation from current status of C sink, to potential C source (House et al. 2010). Carbon stored in soil is lost to the atmosphere in the form of CO_2 from soil organic matter mineralisation (Dawson & Smith 2007) and CH_4 from the anaerobic decomposition of organic matter by methanogenic microbes (Bubier & Moore 1994; Cooper et al. 2014). Emissions of CO_2 are considerably larger than CH_4 emissions, but the global warming potential of CH_4 is 28 times greater than CO_2, making it an important GHG (IPCC 2013).

The effect of climate change on soil C is controversial (Worrall et al. 2004; Davidson & Janssens 2006; Worral & Burt 2007; Clark et al. 2010), but as temperatures rise the rate of organic matter decomposition is expected to increase, potentially resulting in a positive feedback on climate change (Knorr et al. 2005). This effect is also likely to be exacerbated by current land use practices including drainage, grazing and burning (Wallage et al. 2006; Ward et al. 2007 & 2013) and by the effects of nutrient enrichment from nitrogen (N) deposition (Bragazza et al. 2006). Nutrient availability also affects soil C fluxes via influences on plant productivity and inputs of labile C into soil.

Net fluxes of C from soil are affected by numerous environmental factors and ecosystem processes. Soil moisture, temperature and pH have strong controlling effects on emissions of gaseous C from soil, by influencing soil microbial and plant root activity, and the diffusion of gases through soil pores (Smith et al. 2003; Chen et al. 2015). Vegetation composition can also affect rates of both CO_2 and CH_4 emission (Raich & Tufekciogul 2000; Robroek et al. 2015). For CH_4, the effect is direct via the transport of gas through the aerenchymatous tissue of some vascular plants (notably sedges), allowing gaseous exchange with the atmosphere (Joabsson et al. 1999; McEwing et al. 2015). For CO_2, vegetation composition has indirect effects via changes to net C input by plants, variation in decomposition resistance of plant material, rates of root respiration, and influences on soil microclimate and structure (Raich & Tufekciogul 2000). This has significant implications for soil C flux in the context of current environmental change (Berendse et al. 2001; Ward et al. 2013; Xu et al. 2015) and potentially makes forecasting soil C flux highly complex, as all biotic and abiotic factors not only act directly, but also indirectly through the modification of vegetation composition by soil characteristics, and *vice versa* (McEwing et al. 2015).

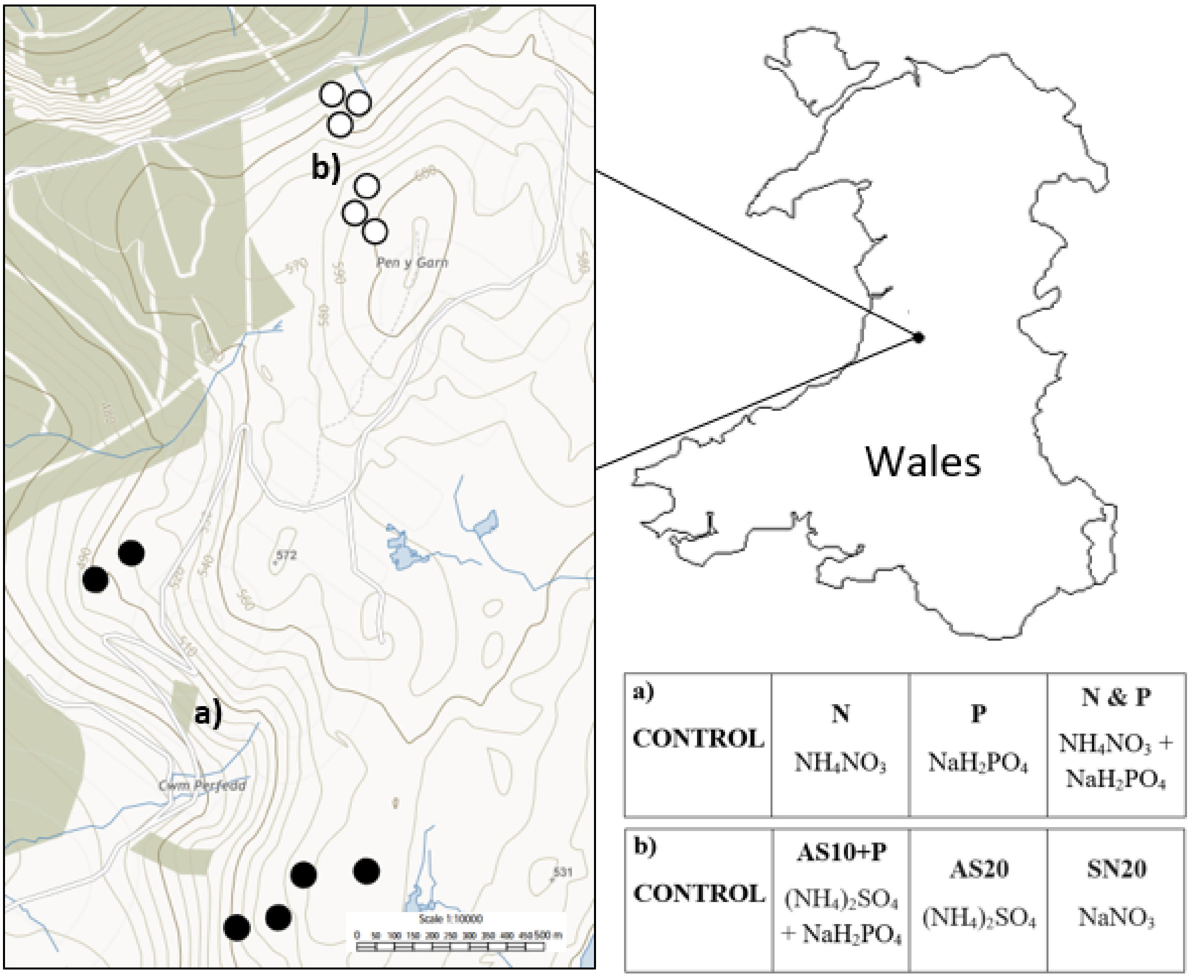
Nitrogen enrichment from pollutant deposition affects ecosystem processes (Magnani et al. 2007; Jones & Power 2012; Southron et al. 2013) and can affect rates of C flux through changes to vegetation and microbial activity (Basiliko et al. 2006; Juutinen et al. 2010; Wu et al. 2015). The effect of N on soil CO_2 flux is controversial, with studies demonstrating an inhibitory (Phillips & Fahey 2007; Janssens et al. 2010; Ramirez et al. 2010) or a stimulatory (Bragazza et al. 2006; Cleveland & Townsend 2006; Zhang et al. 2013) effect. This is potentially the result of differences in C availability (Knorr et al. 2005). In oligotrophic environments with soils of high organic matter content, nutrient enrichment from N deposition is expected to affect soil C storage potential by increasing microbial activity and thus decomposition rates (Bragazza et al. 2006). It is also expected that an increase in N availability will shift ecosystems towards limitation of other nutrients such as phosphorus (P) (Crowley et al. 2012; Peñuelas et al. 2013). Phosphorus limitation may be an important mechanism that constrains productivity in situations of high N deposition; hence, release from this may greatly affect many ecosystem processes and fluxes. Understanding the role of nutrient availability in determining C fluxes and C storage potential is of considerable importance. The availability of reactive N in upland habitats with peaty soils has been increased by anthropogenic sources, and is projected to double in size globally from current levels by 2050 (Galloway et al. 2004; Phoenix et al. 2006). The rate of reactive N input is reportedly in decline in Western Europe (Fowler et al. 2004), but current rates remain higher than the estimated critical load for many upland habitats (RoTAP 2012).

To date, research in this area has focussed on the environmental factors which promote C flux, such as vegetation composition, temperature and soil moisture. Few studies have considered the effect of increased N availability on C flux within upland habitats that have been exposed to N deposition rates near or above the critical load, with none considering the impact of P limitation in this context. This study aimed to establish the effect of N and P enrichment on soil C flux by measuring CO_2 and CH_4 emissions across two randomised block experiments, which have been run in both the short (one year) and longer term (1996 - 2012, with sampling undertaken three years after ceasing N inputs and 15 years after a single P application). We hypothesise that the addition of nutrients (N or P) will increase CO_2 flux (H1) and increase CH_4 flux (H2) at these upland sites, and N and P addition will have stronger stimulation effects than N addition alone on fluxes of CO_2 (H3) and CH_4 (H4). The response will be greatest in treatments where P is added, since plant growth and microbial activity are expected to increase with alleviation from nutrient limitation.

1.2 METHODS

1.2.1 EXPERIMENTAL DESIGN

This study was conducted at two sites: Pen y Garn (PEN - 52^0 37’ N, 3^0 76’ W) and Pwllpeiran (PWL - 52^0 37’ N, 3^0 77’ W) in the Cambrian Mountain range, mid-Wales. The sites were located within 1 km of each other and within an altitude range of 500 - 600 metres a.s.l.. Both sites were on a transition between NVC U4 *Festuca ovina / Agrostis capillaris* grassland and H18 *Vaccinium myrtillus / Deschampsia flexuosa* heath, overlying mixed soils ranging from shallow ferric stagnopodzol to deep peat (Emmett et al. 2007; Phoenix et al. 2012). The mean annual rainfall rate for this location was 1512.2 mm (UK Meteorological Office, no date) and the background N deposition rate was 22 kg N ha^{-1} yr^{-1} (Emmett et al. 2007). Nutrient addition experiments were established at both locations, each consisting of six replicate blocks of four 3 x 3 m plots, in a randomised block design. In the long-term experiment at PEN (Figure 1b), N was added fortnightly between 1996 and 2012, and P was added once in 2000 to the ammonium sulphate 10 kg N ha^{-1} yr^{-1} (AS10+P) treatment. The second experiment, at PWL, was established more recently, with different nutrient addition treatments to better distinguish the effect of P addition by including a P-only treatment (Figure 1a). In both experiments, the addition of N was split to avoid abrupt increases in concentration, with additions of 1/14 of the total dose every two weeks between the months of April – October 2014.



a) CONTROL	N NH$_4$NO$_3$	P NaH$_2$PO$_4$	N & P NH$_4$NO$_3$ + NaH$_2$PO$_4$

b) CONTROL	AS10+P (NH$_4$)$_2$SO$_4$ + NaH$_2$PO$_4$	AS20 (NH$_4$)$_2$SO$_4$	SN20 NaNO$_3$

Figure 1. Experimental treatments at two sites, each replicated six times: black circles and a) PWL, (experiment set up in 2004): CONTROL = no addition; N = ammonium nitrate at 60 kg N ha^{-1} yr^{-1}; P = phosphorus (sodium dihydrogen orthophosphate) at 40 kg P ha^{-1} yr^{-1}; N & P = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} + sodium dihydrogen orthophosphate at 40 kg P ha^{-1} yr^{-1}; white circles and b) PEN (experiment set up in 1996): CONTROL = no addition; AS10+P = ammonium sulphate at 10 kg N ha^{-1} yr^{-1} + phosphorus (sodium dihydrogen orthophosphate) at 20 kg P ha^{-1} yr^{-1}; AS20 = ammonium sulphate at 20 kg N ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20 kg N ha^{-1} yr^{-1}.

At the PWL site, P was added once in April 2014. The PWL site was grazed during the study, at a rate of 1.0 sheep ha^{-1}. The PEN site was not grazed during this study, but had been grazed by sheep between 1990 and 2007 at two levels (1.0 sheep ha^{-1} and 1.5 sheep ha^{-1}). Although there was no grazing at the PEN site between 2007 and 2015, these moorland habitats are resistant to succession and there was no change in habitat type. Three replicate blocks were established in each of two paddocks that had been grazed at the different levels, but the lack of grazing during the intervening seven years had greatly diminished effects of the grazing treatments and differences in previous stocking rate were not taken into account in the design.

1.2.2. VEGETATION ANALYSIS

Vegetation data were collected for PWL in June 2014 and June 2015 and for PEN in June 2015. Cover was recorded visually for each species within each 3 x 3 m plot using the Domin scale and subsequently transformed to percentage cover for statistical analysis (Currall 1987). All plants and bryophytes were identified to species level. Vegetation height was recorded as the average of five measurements to the top of the canopy taken using a metric sward stick, of ca. 1 cm diameter, marked at 0.5 cm intervals (Dennis *et al.* 2005). At PEN, heights were taken from the centre of each plot and then from the mid-point between the centre and each corner. At PWL, heights were recorded from within small grazing exclosures (~1 m^{-2}) that were established within each plot.

1.2.3 SOIL ANALYSIS

Soil samples for both sites were collected in June 2015 from five locations within each treatment plot, using a 20 mm diameter soil corer up to a depth of 20 cm, and bulked together. The samples were air dried and passed through a 2 mm sieve. Soil pH was measured with a Hydrus 400 meter (Fisherbrand, Leicestershire, UK) in a slurry of 10 g fresh soil in 25 mL water. Total C was established by the Dumas combustion method using an elemental analyser (Vario MAX Cube – Elementar Analysensysteme, Hanau, Germany).

1.2.4 SOIL FLUX

Fluxes of CO_2 and CH_4 were measured for the different nutrient addition treatments at both PWL and PEN from September 2014 to August 2015; initially every two months until March 2015, then every month during the growing season (May to August 2015), under dark conditions using a non-steady state, static chamber approach (Livingston & Hutchinson 1995; Parkin & Venterea 2010). Measured CO_2 flux thus represents both soil and plant respiration (ecosystem respiration) but excludes the effects of photosynthesis. Soil collars, 25 cm in diameter, were installed on each treatment in July 2014, two months prior to the first gas flux measurements to reduce disturbance. A soil knife was used to cut through the initial vegetation and topsoil layer, before the collar was inserted to a depth of 7 cm, leaving 5 cm aboveground for chamber attachment. The location of the collar was selected randomly for each treatment square and once installed, vegetation was trimmed from inside the collar and maintained trimmed throughout sampling. Chamber design followed the description by Parkin & Venterea (2010). Each chamber measured 19 cm in height including the soil collar and had an internal volume of 9.33 L. A modified pressure vent tube (Xu *et al.* 2006) was installed to allow internal and ambient air pressure equilibration. Before each measurement, chambers were placed carefully on collars, ensuring a gas tight seal. The soil CO_2 and CH_4 fluxes were calculated based on changes in chamber concentrations over 30 minutes. Measurements were taken for each chamber at 0, 15 and 30 minutes from chamber attachment (Parkin & Venterea 2010). The duration of measurement at each sampling point was 60 seconds. CO_2 and CH_4 concentrations were measured using an LGR™, Ultra-Portable Greenhouse Gas Analyser (Model 915–0011, Los Gatos Research, Palo Alto, CA, USA) with a 1 Hz sampling rate. Chambers were attached to the analyser via inlet and outlet tubing (2 m by 4 mm internal diameter). Chamber internal temperature, ambient air temperature and soil temperature at a depth of 10 cm were also recorded. The rate of change in gas concentration inside the chambers was established with linear regression. Gas flux (CO_2 and CH_4) was then calculated from the rate of gas concentration change using the following equation (McEwing et al. 2015):

$$F_0 = S\,\frac{V\,M\,273.16}{A\,Vm(273.16 + T)}\,60$$

Where:

F_0 = Flux (μg CH_4/CO_2-C m^{-2} hr^{-1})
S = Rate of change in CH_4 and CO_2 concentration (ppm min^{-1})
V = Chamber volume (m^3)
A = Chamber area (m^2)
M = Molecular mass of CH_4/CO_2 (g mol^{-1})
V_m = Ideal gas mole volume (0.0224 m^3 mol^{-1})

Each regression plot was assessed individually using R^2 as an indicator of accuracy, and plots with R^2 >0.7 were accepted for analysis. Low fluxes for CH_4 typically give a low R^2, but should be included to avoid over-estimation of mean flux (Alm et al. 2007), so CH_4 fluxes where R^2 <0.7, but where measurements did not exceed 0.3 ppm, were also retained as zero measurements. Two collars at PWL suffered from excessive water pooling, which visibly affected the vegetation and soil. These were removed from the dataset before analysis. Net ecosystem exchange was not considered as part of this study, so whilst the results demonstrate differences in C flux, primary productivity was not measured and thus the full influence of N and P availability on C budget cannot be determined.

1.2.5 STATISTICAL ANALYSIS

All variables were tested visually for normality and homoscedasticity with Levene's test prior to statistical analysis. Data for CO_2 and CH_4 fluxes were Log (x+1) transformed to meet the assumptions of analyses. Differences in CO_2 and CH_4 flux between treatments were analysed with linear mixed models specifying repeated measures, with treatment as a fixed factor and air temperature as covariate to allow for diurnal/seasonal variance. When significant differences between treatments were detected ($P < 0.05$), post-hoc tests were conducted using LSD pairwise comparisons. The relationship between GHG flux and air temperature was initially tested for significance with simple linear regression before inclusion in the model. Air temperatures were used in the analysis since data for soil temperature were only captured from January onwards, but the two measurements were shown to be correlated (PWL: $R^2 = 0.52$, $P = <0.001$; PEN: $R^2 = 0.81$, $P = <0.001$). Differences in mean annual soil emission of CO_2 and CH_4 between experimental treatments were also investigated with one-way analysis of variance (ANOVAs). LSD pairwise comparisons were used to further investigate individual relationships.

Multiple regression analysis with all-possible-subsets model procedure was used to investigate the relationships between annual average GHG flux and environmental parameters: soil pH, vegetation height, and cover values for each of five plant functional types. All statistical analyses were conducted with SPSS 21.0 (IBM SPSS Statistics for Windows, 2012).

1.3 RESULTS

1.3.1 ENVIRONMENTAL CONTROLS ON CO_2 AND CH_4 FLUX

Over the sampling period, fluctuations were observed for air and soil temperatures in line with seasonal variation. The highest air temperatures were recorded in June for PWL (22.3 °C) and in August for PEN (19.4 °C). Air temperatures were lowest at both locations in January (PWL = 5.0 °C, PEN = 6.2 °C). Soil temperatures were similar with the highest values recorded in August for PWL (13.2 °C) and in July for PEN (12.9 °C) and lowest vales for both in January (PWL = 4.6 °C, PEN = 3.5 °C). Over the twelve months of sampling, the effect of changing air temperature on CO_2 flux was significant for both sites, with positive relationships observed between increasing temperature and CO_2 emission (PWL: $R^2 = 0.68$, $P = <0.001$; PEN: $R^2 = 0.70$, $P = <0.001$, Figure 2). The effect of temperature on CH_4 flux was less consistent; no relationship was observed at PWL ($R^2 = 0.004$, $P = 0.37$), but a significant positive relationship was recorded at PEN ($R^2 = 0.033$, $P = 0.012$).

The results of the multiple regression analysis of effects of vegetation cover values, vegetation height and soil pH on mean annual CO_2 and CH_4 fluxes are shown in Table 1. This analysis revealed significant relationships between soil pH and fluxes for both CO_2 and CH_4 at PWL (Table 1, Figure 3), but other factors were shown to have no effect. At PEN, no environmental factor was shown to have an effect on either CO_2 or CH_4 flux despite the significant differences in vegetation cover between treatments as a result of long-term nutrient addition (Figure 6).

Table 1. Relationships between gas flux and environmental variables with potential influence on C emission.

Site	Variable	Regression model	Degrees of freedom	R^2	*P*
PWL	**CO2**	**pH**	**1,21**	**0.52**	**<0.001**
	CH4	**pH**	**1,21**	**0.25**	**0.019**
PEN	CO2	-	1,23	-	ns
	CH4	-	1,23	-	ns

P-values represent the chance that the regression slope is not different from 0; bold highlights results significant at *P* <0.05. The best fitting regression model using all-possible-subsets regression analysis is shown for each parameter. Variables included in the analysis were: ground cover of each plant functional type (graminoid, cryptogam, dwarf shrub, forbs), vegetation height and soil pH.

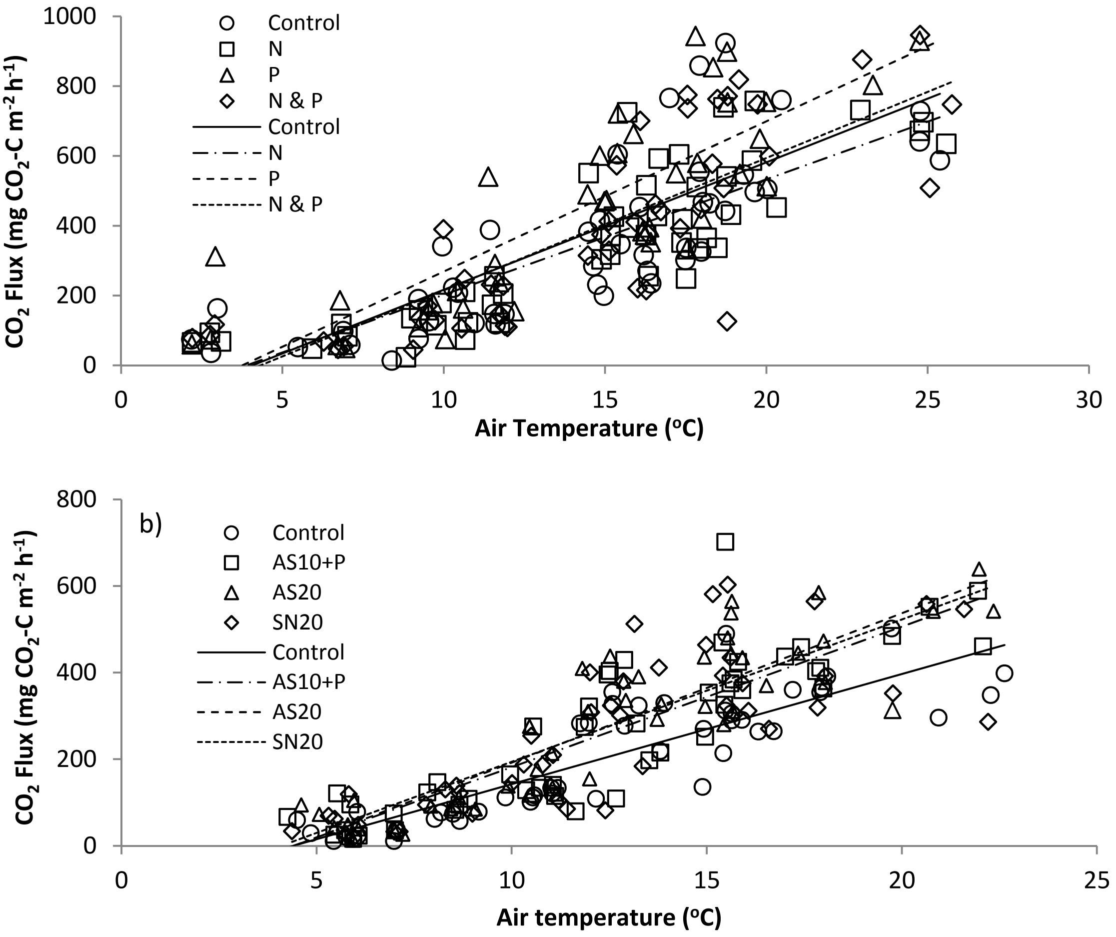


Figure 2. Relationships between CO_2 flux and air temperature at two experimental sites: a) PWL: Control = no nutrient addition (circles); N = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} (squares); P = phosphorus (sodium dihydrogen orthophosphate) at 40 kg P ha^{-1} yr^{-1} (triangles); N & P = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} + phosphorus at 40 kg P ha^{-1} yr^{-1} (diamonds); and b) PEN: Control = no nutrient addition (circles); AS10+P = ammonium sulphate at 10 kg N ha^{-1} yr^{-1} + phosphorus at 20 kg P ha^{-1} yr^{-1} (squares); AS20 = ammonium sulphate at 20 kg N ha^{-1} yr^{-1} (triangles); SN20 = sodium nitrate at 20 kg N ha^{-1} yr^{-1} (diamonds).

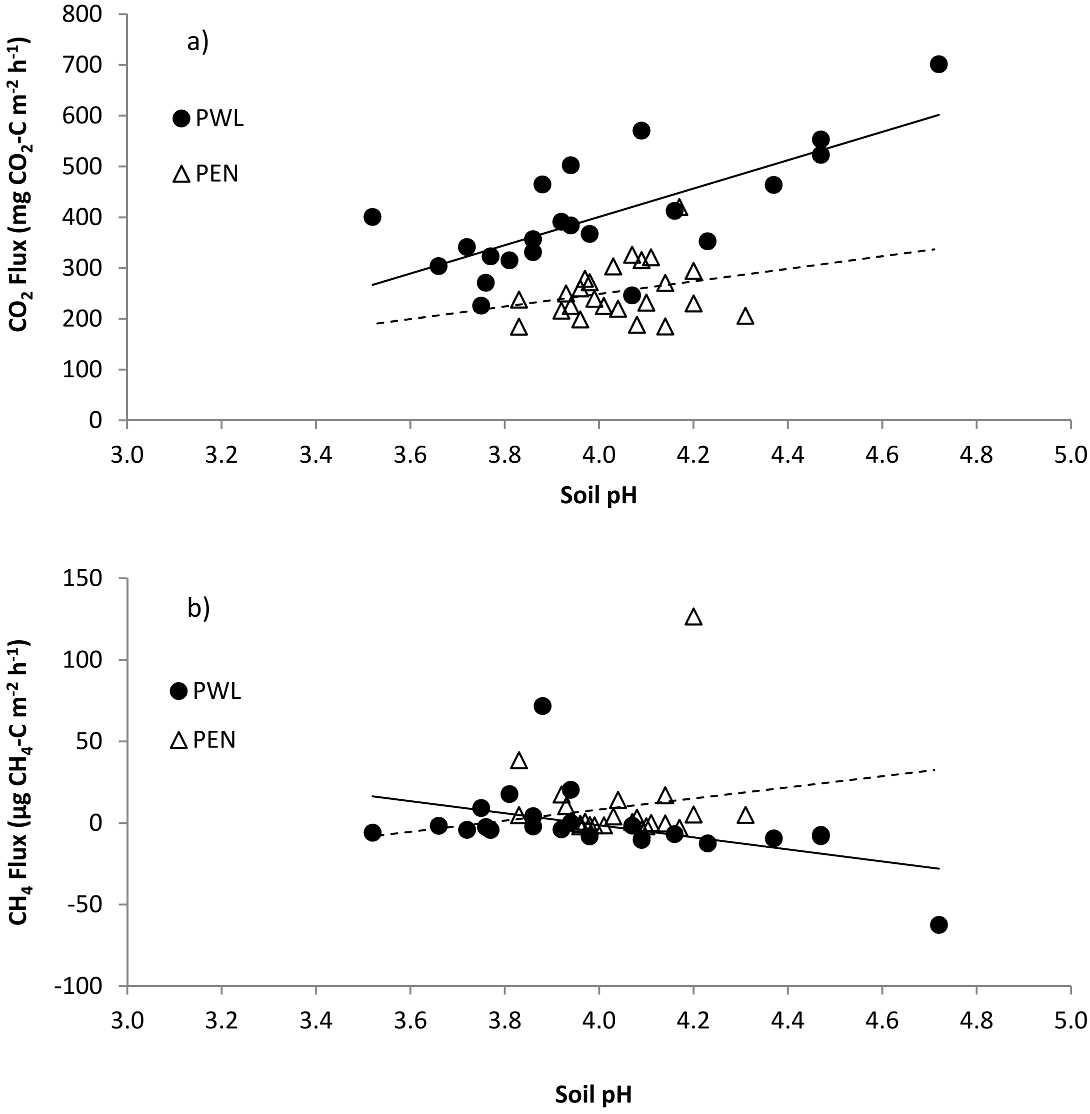


Figure 3. The relationship between soil pH and a) CO_2 flux and b) CH_4 flux at two sites: PWL (black circles) and PEN (white triangles). Solid lines indicate significant relationships, dashed lines non-significant relationships.

1.3.2 EFFECTS OF NUTRIENT ADDITION ON CO_2 FLUXES

Differences were observed in the CO_2 fluxes between nutrient addition treatments at both sites, but with differences between sites in the size of response observed. For PWL, the between-treatment differences (Table 2, Figure 4a) were shown by *post hoc* analysis to be driven by the largest mean annual flux recorded for the P addition treatment (464 mg CO_2-C m^{-2} h^{-1}). The P treatment had consistently higher CO_2 flux in all but the first month sampled (September) (Table 2, Figure 4a). The N addition treatment had the lowest mean annual flux (353 mg CO_2-C m^{-2} h^{-1}) and was consistently the lowest flux recorded across the sampling period (in all months except May). The CO_2 fluxes in the control and N + P treatments had similar mean annual fluxes (386 and 413 mg CO_2-C m^{-2} h^{-1} respectively). For PEN, *post hoc* analysis revealed the differences between treatments (Table 2,

Figure 4b) to be due to the lowest mean annual flux recorded, which was the control (205 mg CO_2-C m^{-2} h^{-1}). The AS10+P, AS20 and SN20 treatments had similar mean annual fluxes (261, 277 and 274 mg CO_2-C m^{-2} h^{-1} respectively). The differences observed between treatments at PEN suggest that nutrient enrichment in the long term significantly increases CO_2 flux relative to control, but there were no statistically significant differences among the nutrient addition treatments, suggesting limited influence of the P added to the AS10+P treatment on CO_2 flux 15 years after application.

Table 2. Summary of results from Linear Mixed Models describing soil CO_2 and CH_4 flux responses to nutrient addition treatments, with treatment as fixed factor and air temperature as covariate.

Site	**Variable**	**Factor**	**Degrees of freedom**	***F***	***P***
PWL	CO_2	**Treatment**	3, 171	**3.69**	**0.013**
		Air temperature	1, 171	**422.32**	**<0.001**
	CH_4	**Treatment**	3, 171	**2.86**	**0.041**
		Air temperature	1, 171	0.60	0.439
PEN	CO_2	**Treatment**	3, 187	**4.63**	**0.004**
		Air temperature	1, 187	**461.23**	**<0.001**
	CH_4	**Treatment**	3, 187	**4.22**	**0.006**
		Air temperature	1, 187	**6.75**	**0.01**

P-values refer to of the likelihood that the coefficient is zero, bold highlights results significant at P <0.05.

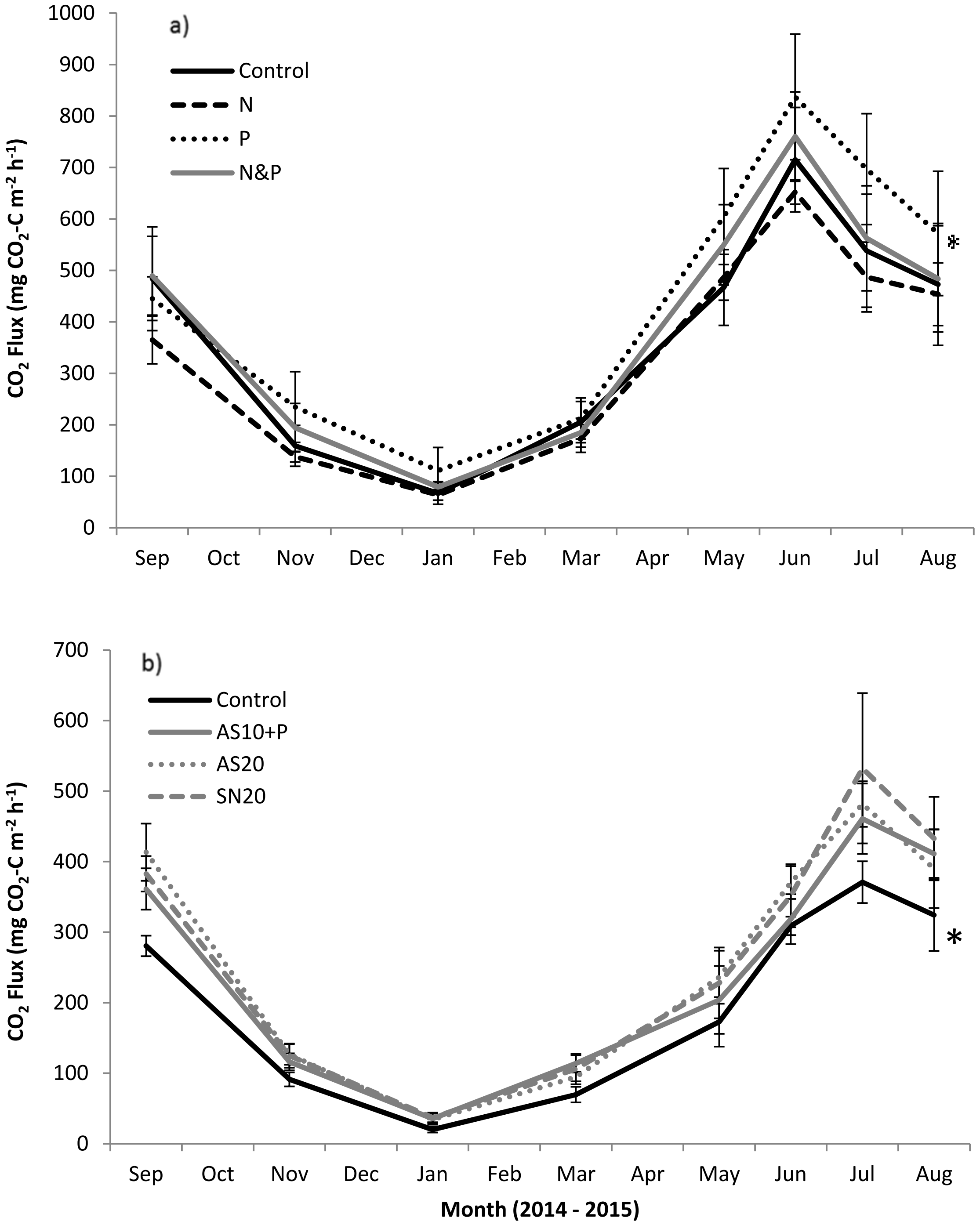


Figure 4. Net CO_2 emission fluxes for a) PWL and b) PEN. Error bars denote standard error. * indicates treatment with significant difference as determined by *post hoc* pairwise comparison (LSD). PWL: Control = no nutrient addition; N = ammonium nitrate at 60 kg N ha^{-1} yr^{-1}; P = phosphorus (sodium dihydrogen orthophosphate) at 40 kg P ha^{-1} yr^{-1}; N & P = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} + phosphorus at 40 kg P ha^{-1} yr^{-1}. PEN: Control = no nutrient addition; AS10+P = ammonium sulphate at 10 kg N ha^{-1} yr^{-1} + phosphorus at 20 kg P ha^{-1} yr^{-1}; AS20 = ammonium sulphate at 20 kg N ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20 kg N ha^{-1} yr^{-1}.

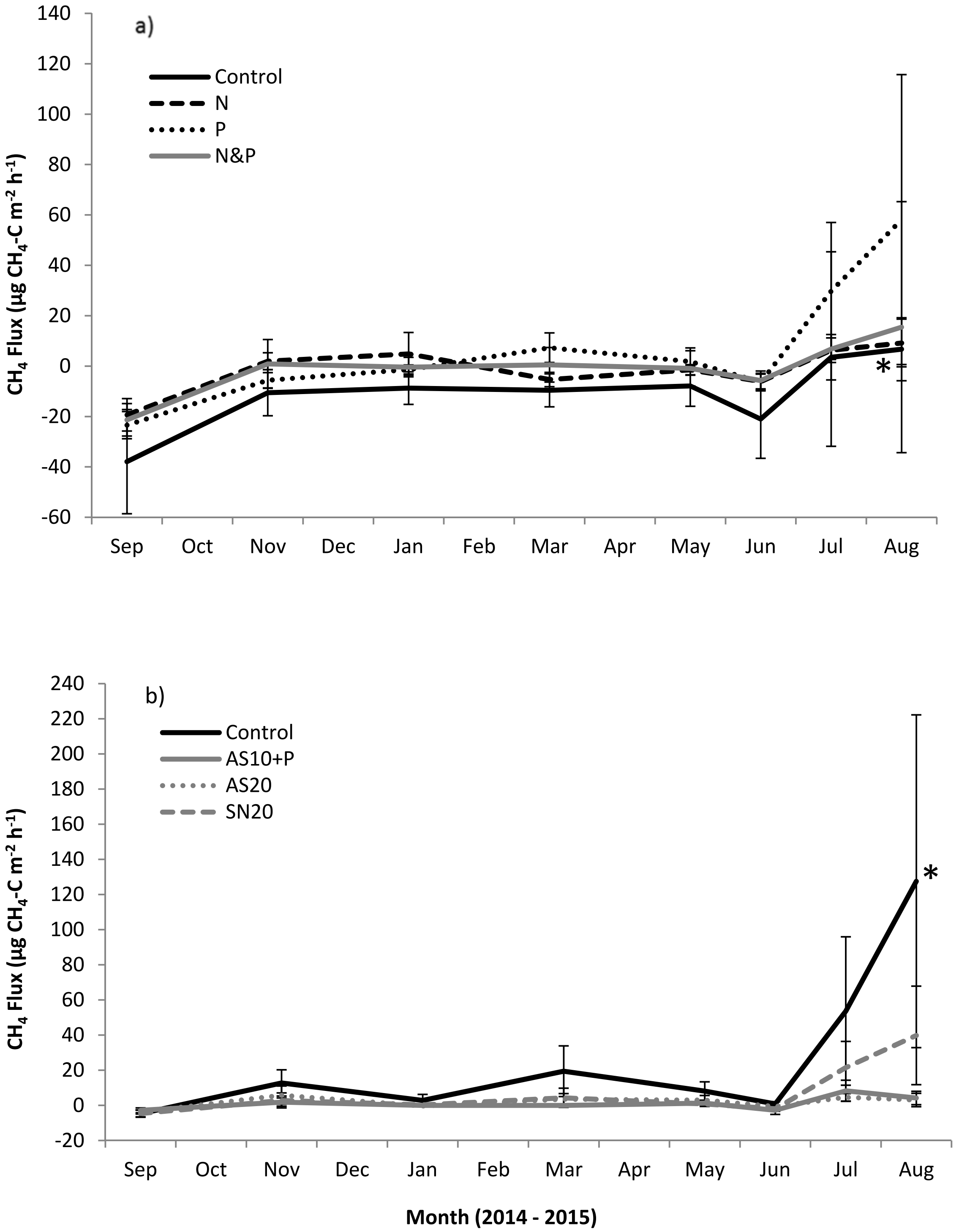


Figure 5. Effects of nutrient addition treatments on net methane emissions for a) PWL and b) PEN sites. Error bars denote standard error. * indicates treatment with significant difference as determined by *post hoc* pairwise comparison (LSD). PWL: Control = no nutrient addition; N = ammonium nitrate at 60 kg N ha^{-1} yr^{-1}; P = phosphorus (sodium dihydrogen orthophosphate) at 40 kg P ha^{-1} yr^{-1}; N & P = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} + phosphorus at 40 kg P ha^{-1} yr^{-1}. PEN: Control = no nutrient addition; AS10+P = ammonium sulphate at 10 kg N ha^{-1} yr^{-1} + phosphorus at 20 kg P ha^{-1} yr^{-1}; AS20 = ammonium sulphate at 20 kg N ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20 kg N ha^{-1} yr^{-1}.

1.3.3 EFFECTS OF NUTRIENT ADDITION ON CH_4 FLUXES

The extent to which differences in CH4 fluxes could be attributed to treatment effects varied according to experimental site. At PWL there were significant differences between treatments (Table 2, Figure 5a), independent of seasonal variations in temperature (Table 2), which *post hoc* tests revealed to be driven by the difference in CH_4 emissions between the control (no addition) and nutrient addition treatments (N, P and N + P). At this site, CH_4 uptake was greatest in control plots, with this treatment behaving as a small sink (on average -10.68 μg CH_4-C m^{-2} h^{-1}), only emitting CH_4 during the last two months (June and August), at rates just above zero μg C CH_4 m^{-2} h^{-1}. The N, P and N + P treatments were highly variable in emission rate, with fluctuations recorded across the sampling range. The P addition treatment had the largest mean annual flux (9.00 μg CH_4-C m^{-2} h^{-1}), which can chiefly be attributed to an increase in emission rate during the last two months (Figure 5a). The N and N + P treatments were intermediate in response, with emission rates just below zero (N = -1.05 μg CH_4-C m^{-2} h^{-1}, N + P = -0.77 μg CH_4-C m^{-2} h^{-1}) on a mean annual basis ($P < 0.05$). At PEN there were also significant differences observed between treatments (Table 2, Figure 5b), which *post hoc* analysis revealed to be likewise driven by the difference between control and all nutrient addition treatments. However, at this site the CH_4 flux was significantly higher in control plots than nutrient added treatments, with mean annual emissions of 27.64 μg CH_4-C m^{-2} h^{-1} ($P < 0.01$). All of the nutrient addition treatments at PEN otherwise had similar flux rates for the full sampling range, until the last two months, when the CH_4 flux from the sodium nitrate treatment (SN20) increased comparatively (Figure 5b).

1.3.4 EFFECTS OF NUTRIENT ADDITION ON VEGETATION COMPOSITION

The addition of nutrients resulted in significant differences in the observed vegetation cover, but only after sufficient time had elapsed allowing vegetation community modification. At PWL, where nutrients were added in the short term only, no differences in vegetation cover were observed. At PEN, long-term nutrient additions resulted in greater graminoid cover and lesser cryptogam cover where N alone was added and greater cryptogam and lesser graminoid cover in treatments where P was added (AS10+P). Control plots were intermediate in composition (Figure 6).

PWL PEN

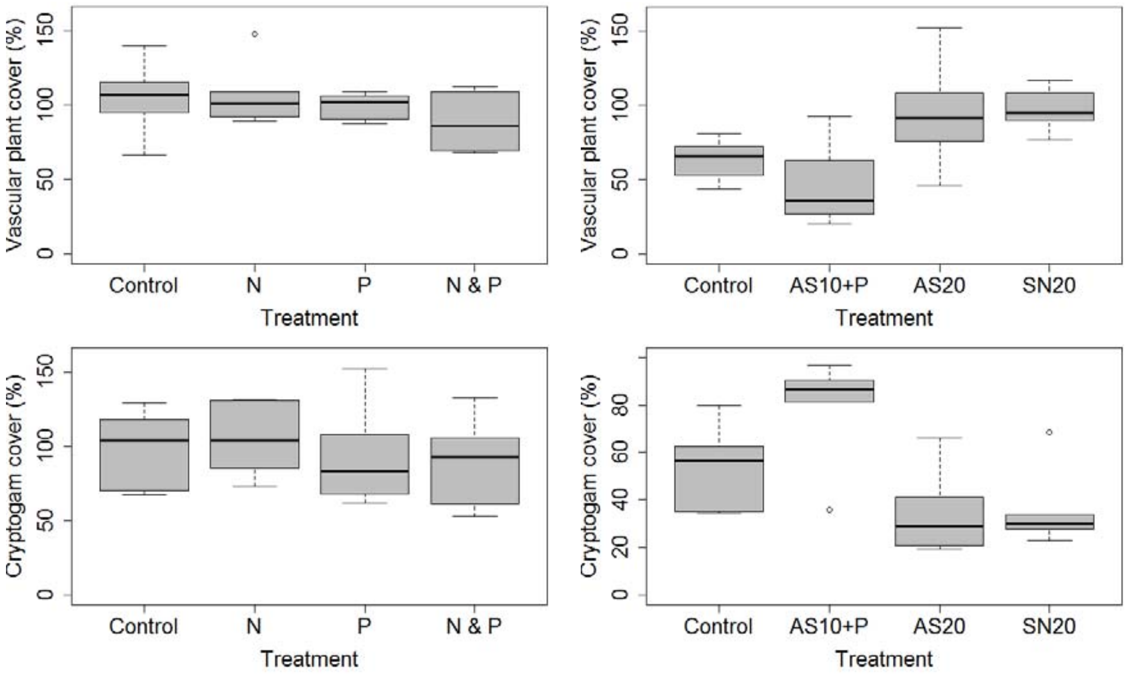


Figure 6. Ground cover of vascular plants and cryptogams recorded for nutrient addition treatments at two sites, PWL (L-hand plots) and PEN (R-hand plots). PWL: Control = no nutrient addition; N = ammonium nitrate at 60 kg N ha^{-1} yr^{-1}; P = phosphorus (sodium dihydrogen orthophosphate) at 40 kg P ha^{-1} yr^{-1}; N & P = ammonium nitrate at 60 kg N ha^{-1} yr^{-1} + phosphorus at 40 kg P ha^{-1} yr^{-1}. PEN: Control = no nutrient addition; AS10+P = ammonium sulphate at 10 kg N ha^{-1} yr^{-1} + phosphorus at 20 kg P ha^{-1} yr^{-1}; AS20 = ammonium sulphate at 20 kg N ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20 kg N ha^{-1} yr^{-1}. Box midline indicates median, box edges indicate interquartile range. Whiskers indicate range of data within 1.5 x the interquartile range; points indicate data outside 1.5 x the interquartile range.

1.4 DISCUSSION

The addition of N and P had significant influence on CO_2 and CH_4 fluxes, with differences in the direction and magnitude of the effects between sites as a consequence of duration of nutrient addition treatment. In the short-term trial, N addition inhibited CO_2 and CH_4 flux, whereas in the long-term trial, N addition significantly increased CO_2 emissions but inhibited CH_4 flux. The addition of P significantly increased CO_2 and CH_4 flux in the short-term trial, but this effect was reduced in the long-term trial, after 14-15 years since P addition.

1.4.1 NUTRIENT ADDITION EFFECTS ON CO_2 FLUXES

The addition of N and P influenced fluxes of CO_2, however there were substantial differences in effect between treatments, and also differences in response between short-term (PWL = 1 year for N & P) and long-term nutrient additions (PEN = additions between 1996 – 2012, sampling undertaken three years after the cessation of N addition and 15 years after a single P application). At PWL, the addition of P increased CO_2 flux, which supported hypothesis H3 and is in line with findings from similar studies in other environments (Cleveland & Townsend 2006; Liu et al. 2013). Phosphorus limitation is a significant mechanism constraining ecosystem processes, particularly in systems suffering from the effects of chronic N deposition (Cleveland et al. 2011; Crowley et al. 2012). The addition of P stimulates decomposition, with observed rises in CO_2 flux associated with increases in heterotrophic (bacteria and fungi) biomass and activity, and thus respiration (Liu et al. 2012), rather than through increased fine-root biomass (Cleveland & Townsend 2006). Phosphorus addition results in the modification of microbial community structure and reduces the ratio between Gram-positive and Gram-negative bacteria, resulting in more copiotrophic communities (Fanin et al. 2015). At PEN, the role of P was less clear. The CO_2 flux in the N + P treatment (AS10+P) was larger than in the control plots, but was mostly lower across the sampling period than CO_2 fluxes from the two N-only treatments (AS20 and SN20). Phosphorus is highly persistent in soil (Nye & Tinker 1977), and was presumably responsible for driving shifts observed in vegetation species composition (Figure 6), thus the comparatively weak effect of P on CO_2 flux at this site was unexpected. The N-only treatments received N at a higher rate than the AS10+P treatment, and associated larger CO_2 fluxes may reflect the effect of greater N availability. In addition, while the effects of P on plant species composition were still visible at the site, P may no longer have been stimulating plant production of labile C. Liu et al. (2013) observed a diminishing effect of P on microbial biomass after four years, which was attributed to C limitation, where the exhaustion of available soil C had a constraining effect on respiration (Fanin et al. 2015).

The addition of N had an inhibitory effect on soil CO_2 flux at PWL and refutes hypothesis H1 that nutrient addition would increase CO_2 efflux. Similar inhibition of decomposition by N has been found in other studies (Ramirez et al. 2010; Chen et al. 2015), and may result from high N availability. In soils where N is not a limiting factor for microbial growth and activity, the addition of N can constrain organic matter decomposition (Janssens et al. 2010). In such instances, reductions in CO_2 flux may be driven by shifts in C allocation from belowground to aboveground biomass (Litton et al. 2007), which reduces rhizosphere and microbial respiration (Phillips & Fahey 2007; Bae et al. 2015). The background N deposition for these sites is 22 kg N ha^{-1} yr^{-1} (Emmett et al. 2007), which is greater than the critical load limit for this habitat (10 – 15 kg N ha^{-1} yr^{-1}; APIS 2014), suggesting that these soils are unlikely to be N limited. In addition, reductions in soil pH associated with N addition potentially have a limiting effect (Chen et al. 2015).

This contrasts with the effects observed at PEN, where N addition treatments had higher CO_2 flux in comparison to control (no addition) treatments, which does support hypothesis H1. PEN was sampled three years after ceasing N addition, which could indicate that inhibitory effects were reduced due to N losses from leaching in the intervening three years. This explanation was deemed unlikely however, as the size and activity of microbial populations exposed to N enrichment can remain elevated 6–8 years after cessation of additions, suggesting a prolonged effect on the rate of nutrient cycling (Power et al. 2006). Instead, the higher CO_2 flux where N was added is most likely the result of greater soil organic matter decomposition from increased microbial abundance and activity (Mack et al. 2004;

Bragazza et al. 2006), and by reduced production of more decomposition resistant species (Bragazza et al. 2012). This influence was not translated to a reduction in soil C levels in N treatment plots however; these plots in fact had the highest soil C content, albeit not significantly greater than control (Stiles et al. 2017). This suggests a potential balance between increased rates of both plant production and decomposition with greater N availability (Mack et al. 2004).

The variability observed in CO_2 flux between sites with N enrichment could be explained by differences in the N addition rate, which at PWL was three times the maximum annual application rate at PEN. Janssens et al. (2010) demonstrated that the inhibitory effect of N on soil CO_2 flux was larger in sites receiving N at dose rates greater than 50 kg ha^{-1} yr^{-1}, which may account for some of the between-site disparity. The inhibitory effect of N at PWL apparently offset the stimulatory effect of P, in that the CO_2 flux response to N + P addition was intermediate between responses to P and N addition. Thus, although the results were not consistent with a general stimulation of CO_2 efflux by nutrient addition (H1), there was support for hypothesis H3 that P would have a greater stimulating effect.

1.4.2 NUTRIENT ADDITION EFFECTS ON CH_4 FLUXES

The addition of N and P altered CH_4 flux, although different effects were observed in the short term after enrichment (PWL) and after a longer period (PEN). The CH_4 flux results represent net emissions, and effects on CH_4 production and oxidation cannot be distinguished. At PWL, all nutrient addition treatments had less negative net CH_4 emissions than the control, supporting hypothesis H2. The less negative net emissions with N addition are presumably due to decreases in the rate of CH_4 oxidation (Aerts & Toet 1997; Aerts & Caluwe 1999). This is primarily caused by competition for the CH_4 mono-oxygenase enzyme, which is affected when N addition increases rates of nitrification, inhibiting CH_4 oxidisation rate and reducing the amount of methane consumed by methanotrophs (Bodelier 2011). Phosphorus addition also increased the rate of emission for CH_4, which supports hypothesis H4, but is contrary to similar research conducted in other environments (Zhang et al. 2011; Song et al. 2012). These studies attributed the effects observed to the inhibition of methanogenesis, the stimulation of methanotrophic potential, and increased plant growth and water uptake reducing soil water content and thus increasing methanotrophy through greater aeration. This last effect would seem unlikely to operate in the PWL and PEN experiments because these are rather wet sites and the treatments would not be expected to increase soil aeration greatly, even with the potentially augmented growth associated with P enrichment. The stimulation of methanogenesis is likely to be the principal mechanism and has been previously observed for some peat types (Keller et al. 2006), which suggests the methanogen community at PWL is P limited. Further tests would be necessary to rule out any inhibitory effect of P on methane oxidation, but this is beyond the scope of the current study.

At PEN, the highest CH_4 flux recorded was in the control, which was contrary to hypothesis H2 that nutrient addition would stimulate CH_4 fluxes. Fluxes of CH_4 from the nutrient addition treatments remained near zero for the majority of the sampling period until the last two months (July and August), when the fluxes from the sodium nitrate treatment (SN20) increased substantially. Nitrate inhibits CH_4 emission, but only in very high concentrations (Bodelier & Laanbroek 2004), whereas ammonium has been shown to inhibit CH_4 emission more strongly (Crill et al. 1994). This may explain why emissions were consistently inhibited in the ammonium treatments (AS10+P and AS20) in contrast to the spike in emission observed in the nitrate treatment towards the end of the study period. Overall, N addition inhibited CH_4 emission, which can be attributed to N-induced increases in population size and activity of methane-oxidising bacteria (Bodelier & Laanbroek 2004). The addition of P (AS10+P) had no obvious effect at PEN, 15 years after application, contrary to the H4 hypothesis. As with the CO_2 flux results, this was unexpected. Whilst the exact mechanisms remain unclear, the composition of the microbial community may have changed over the intervening years (as with the diminishing effect of P over time on CO_2 emission, discussed above) which may have reduced the size of any initial effect. This may also account for the substantial differences observed in the results of the two experiments.

1.4.3 ENVIRONMENTAL CONTROLS ON CARBON FLUXES

Temperature influenced the rate of CO_2 flux at both PWL and PEN, which is consistent with other studies (Dorrepaal et al. 2009; Briones et al. 2010; Imer et al. 2013). Higher temperatures allow increased rates of soil decomposition and root respiration, which are temperature-dependent chemical and biochemical reactions (Knorr et al. 2005; Davidson & Janssens 2006). The role of temperature in controlling CH_4 emission is less definite and was variable between sites. At PEN, there was a positive effect of temperature on CH_4 flux, whereas at PWL, no effect was observed. The effect of temperature on CH_4 production is reported to be variable and driven by the influence of site-specific factors such as differences in soil type (Chin et al. 1999; Van Winden et al. 2012). The effect of temperature is likely to be closely linked with that of soil moisture and aeration (Smith et al. 2003). If high temperatures relate to drought and therefore dry soil, it would be expected that CH_4 emission would be reduced, but CO_2 emission would increase (Sundh et al. 2000). Plant functional type composition and above ground biomass quantity did not influence CO_2 or CH_4 fluxes, which is contrary to previous studies (McNamara et al. 2008; Green & Baird et al. 2012; Cooper et al. 2014; McEwing et al. 2015). This was also contrary to expectation, as differences in vegetation composition exist between treatments, particularly at PEN, where long-term nutrient additions have resulted in divergent vegetation composition (Figure 6). Nitrogen addition (AS20 and SN20 treatments) has driven an increased ground cover of graminoid species, whereas N + P addition (AS10+P) has driven an increased ground cover of cryptogam species. Vegetation impacts on CH_4 flux are particularly associated with *Eriophorum* spp. presence and cover (Green & Baird et al. 2012), as these sedges have aerenchymatous tissue, which can facilitate the transport of CH4 from the anaerobic zone to the atmosphere, and actively produces substrates that encourage methanogenesis (Cooper et al. 2014). The relative scarcity of these species at either site could potentially account for the limited effect of vegetation composition changes on CH_4 flux.

The positive relationship for CO_2, and negative relationship for CH_4, observed between fluxes and soil pH at PWL, can be explained by changes in microbial activity. Soil acidity may be increased by N enrichment (Bobbink et al. 2010; Phoenix et al. 2012), an effect which is reported to have a stronger controlling effect on C flux than stimulation by increased N availability (Chen et al. 2015). Soil pH can affect the activity rate and composition of microbial communities, with decreases of 1.5 pH units shown to reduce activity by 50% (Fernández-Calviño & Bååth 2010). Recruitment of bacterial species more tolerant of lower pH ultimately modifies the community, but with an intervening lag in activity (Rousk et al. 2010), a hysteresis which may explain the observed reduction in the CO_2 emission. Low soil pH may reduce CH_4 emissions through inhibition of methanotrophic bacterial communities (Dedysh 2002). These effects potentially explain some of the differences observed between the two sites, in that changes to pH driven by recent nutrient additions at PWL may have resulted in short-term fluctuations in microbial activity. This effect would be expected to reduce over time as the system shifts to a new equilibrium, as in the longer-term experiment at PEN. The absence of any association with other environmental variables at PEN indicates that, for this study at least, the availability of nutrients over an extended period (even three years after application for N and 15 years after application for P) has a greater influence on CH_4 flux rate than other background environmental factors.

1.5 CONCLUSION

The addition of N and P had significant effects on CO_2 and CH_4 fluxes but the direction and magnitude of effects were different in the two experiments, most probably due to the difference in duration of additions between the two sites. The CO_2 flux was inhibited by recent additions of N, but plots that had received prolonged N additions up until three years previously showed considerably increased CO_2 emissions. The long-term effects of P addition are less clear. In the short term, P stimulated CO_2 emissions via release from nutrient limitation, but this effect appeared to diminish with time, perhaps due to a decrease in readily available C substrates. This implies a negative effect of P addition on soil C storage, although this may be offset at some sites by plant productivity increases. The emission rate of CH_4 for treatments where nutrients were added was of similar size between sites, despite differences in the CH_4 flux observed in control treatments at either site. This demonstrated a similar pattern of modification by nutrient addition, but with different factors controlling the response relative to either control. The differences between emission rates in control (no addition) treatments at the two sites indicates the influence of other site characteristics on CH_4 flux rate.

The contrast in results from the two experiments demonstrates the differences between initial ecosystem responses to nutrient addition and responses after ecosystem processes and microbial assemblages have had sufficient time to adjust to more nutrient-rich conditions. Perturbation in ecosystems often drives gradual change, and many experiments do not last long enough for the system to reach a new equilibrium (Bubier et al. 2007; Vitousek et al. 2008). In this study the differences in response between experimental additions in the short and long term were considerable, which has important implications for the use of short-term evidence to infer responses of C storage and other ecosystem processes to continuing atmospheric N pollution, or P additions that persist in the soil. Short-term studies investigating impacts of nutrient enrichment may misrepresent longer-term ecosystem responses.

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