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Short communication

Long-term interactive effects of N addition with P and K availability on N status of *Sphagnum*

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Abstract

Little information exists concerning the long-term interactive effect of nitrogen (N) addition with phosphorus (P) and potassium (K) on *Sphagnum* N status. This study was conducted as part of a long-term N manipulation on Whim bog in south Scotland to evaluate the long-term alleviation effects of phosphorus (P) and potassium (K) on N saturation of *Sphagnum* (*S. capillifolium*). On this ombrotrophic peatland, where ambient deposition was 8 kg N ha^{-1} yr^{-1}, 56 kg N ha^{-1} yr^{-1} of either ammonium (NH_4^+, N_{red}) or nitrate (NO_3^-, N_{ox}) with and without P and K, were added over 11 years. Nutrient concentrations of *Sphagnum* stem and capitulum, and pore water quality of the *Sphagnum* layer were assessed. The N-saturated *Sphagnum* caused by long-term (11 years) and high doses (56 kg N ha^{-1} yr^{-1}) of reduced N was not completely ameliorated by P and K addition; N concentrations in *Sphagnum* capitula for N_{red} 56PK were comparable with those for N_{red} 56, although N concentrations in *Sphagnum* stems for N_{red} 56PK were lower than those for N_{red} 56. While dissolved inorganic nitrogen (DIN) concentrations in pore water for N_{red} 56PK were not different from N_{red} 56, they were lower for N_{ox} 56PK than for N_{ox} 56 whose stage of N saturation had not advanced compared to N_{red} 56. These results indicate that increasing P and K availability has only a limited amelioration effect on the N assimilation of *Sphagnum* at an advanced stage of N saturation. This study concluded that over the long-term P and K additions will not offset the N saturation of *Sphagnum*.

Keywords: Manipulation experiment; N deposition; peatland; *Sphagnum*; phosphorus and potassium interaction

Capsule: *Over the long-term P and K additions will not offset the N saturation of Sphagnum.*

Introduction

There has been widespread concern over the effects of increasing N deposition on peatland ecosystems which are adapted to low nutrient inputs and therefore sensitive to increased N deposition (Bobbink et al., 1998). *Sphagnum* moss, a keystone peatland species, is especially sensitive to increasing N availability because of its efficient interception of incoming N (Van Breemen, 1995; Bobbink et al., 1998). Field (Lamers et al., 2000; Bragazza et al., 2005; Limpens et al., 2011; Harmens et al., 2014) and manipulation studies (Berendse *et al*., 2001; Nordbakken *et al*., 2003; Granath *et al*., 2009; Sheppard et al., 2013; Chiwa et al., 2016b) have been conducted to evaluate the effects of increased N deposition on *Sphagnum* in bog peatlands. It has been found that increases in N deposition enhanced tissue N concentration in *Sphagnum* (Berendse *et al*., 2001; Heijmans *et al*., 2001; Nordbakken *et al*., 2003; Tomassen *et al*., 2003; Granath *et al*., 2009; Fritz *et al*., 2012; Chiwa et al. 2016b) and eventually led to N saturation of *Sphagnum*, defined as an excess of N supply over N demands of plants, resulting in increased inorganic N leakage to the rizosphere (Limpens *et al*., 2003; Bragazza and Limpens, 2004; Limpens *et al*., 2004; Limpens & Berendse, 2003; Chiwa et al., 2016b; Manninen et al., 2016).

Many studies have documented that N deposition can induce P limitation in forests (Gress et al., 2007; Braun et al., 2010; Blanes et al., 2013; Chiwa et al., 2016a; Li et al., 2016) and wetlands (Bragazza et al., 2004; Limpens et al., 2004; Li et al., 2016). Phosphorus (P) and potassium (K) availability is a major factor determining the impact of N deposition on *Sphagnum* growth in bogs (Hoosbeek et al., 2002; Limpens et al., 2004), as it can enhance growth leading to growth dilution of nutrients. Therefore, we need to understand how elevated N deposition interacts with P and K availability to affect the nutrient status of *Sphagnum*.

In many N manipulation studies, however, little information exists concerning the interactive effect of N with the availability of other growth limiting nutrients such as P and K. Previous studies, based on < 3 years of treatment, have shown that P and K addition can alleviate the adverse effects of elevated N deposition on *Sphagnum's* physiological status, and can have positive effects on N assimilation (processing and incorporation of N leading to decreased inorganic N leakage to the rhizosphere) (Limpens et al., 2004), growth (Limpens et al., 2004; Carfrae et al., 2007; Lund et al., 2009; Kivimäki, 2011; Fritz et al., 2012) and cover (Pilkington et al., 2007). However, the long-term interactive effects of P and K on the N status of *Sphagnum* have not been examined for N manipulation sufficient to cause N saturation. Xing et al (2010) examined the effects of 64 kg N ha^{-1} yr^{-1} (NH_4NO_3) with additional P and K for 7 years, but not without P and K addition. Therefore, long-term P and K effects on the alleviation of N saturation of *Sphagnum* exposed to high levels of N deposition need to be clarified. In addition, since N deposition contains two forms of mineral N in varying proportions (Stevens *et al*., 2011), we also need to understand the respective effects of reduced (NH_4^+) versus oxidized (NO_3^-) N with P and K addition on the alleviation of the N saturation of *Sphagnum* moss. The alleviation by P and K addition may vary with N form.

The objective of this study is to evaluate the alleviation effects of P and K availability on N saturation of *Sphagnum* (*S. capillifolium*) in response to increasing availability of oxidized and reduced N chemical forms. In addition to N, P and K are also limiting in these peatland ecosystems (Sheppard et al., 2004). We therefore hypothesized that supplementing N additions with these potentially growth limiting nutrients would reduce the likelihood of N accumulation, reduced growth and associated phytotoxicity.

2. Materials and methods

2.1. Study Site

This study was conducted at Whim bog (282 m a.s.l., 3°16’W, 55° 46’N) located in the Scottish Borders, 30 km south of Edinburgh, Scotland where a fertilization experiment on 3-6 m of deep peat using N, P, and K has been conducted since 2002. *Calluna vulgaris, Eriophorum vaginatum*, *Sphagnum capillifolium*, *Hypnum jutlandicum*, *Pleurozium schreberi* and *Cladonia portentosa* are the most common species on this bog and are representative of similar habitats through the northern hemisphere (Gore, 1983). There has been no active management for at least 70 years. Detailed information on meteorological parameter and atmospheric N deposition at this study site were given in Chiwa et al. (2016b).

2.2. Treatments

The five different treatments (NH_4^+, NH_4^+ + PK, NO_3^-, NO_3^- + PK, and control) have been applied on each of five 12.8 m^2 circular plots. Four replicates were conducted for each of the five treatments. Background N deposition is *ca.* 8 kg N ha^{-1} yr^{-1} (Leith et al., 2004; Sheppard et al., 2004). NH_4Cl and $NaNO_3$ were used as NH_4^+ (referred to as N_{red}) and NO_3^- (referred to as N_{ox}) treatments, respectively. The dose was 56 kg N ha^{-1} yr^{-1} and solution concentration was 4.0 mM. Potassium hydrogen phosphate (K_2HPO_4) was supplied in a 1:14 and 1:5.5 mass ratio for P and K, respectively to N was used as P and K treatments (4 kg P ha^{-1} yr^{-1} and 11.5 kg K ha^{-1} yr^{-1} for P and K, respectively).

Rainwater only was provided as a control. The current maxima are around 40 kg N ha^{-1} yr^{-1} based on measurements in China (Song et al., 2017), up to 50 kg N ha^{-1} yr^{-1} (Wang et al., 2013) or even up to 100 kg N ha^{-1} yr^{-1} (Pan et al., 2012). Historically, N deposition was significantly higher than now, especially in Europe. Examples can be found up to 44 kg N ha^{-1} yr^{-1} (Stevens et al., 2010), 40-80 kg N ha^{-1} yr^{-1} (van Breeman and Dijk, 1988), and up to 75 kg N ha^{-1} yr^{-1} (Dise and Wright, 1995). However, all of these refer to measurements made in relatively unpolluted conditions, and do not reflect N deposition close to point sources (e.g. feedlots) where ecological effects are likely, and N deposition is much greater. P and K these were added in a 1:14 ratio to N, as found in amino acids to ensure sufficiency for growth (Speppard et al., 2004), rather than simulate their levels in deposition.

The mist treatments of fine rain droplets were supplied from a central spinning disc on a plot. To avoid contamination from adjacent plots, plots were 3 m apart. To simulate real world conditions, treatments (*ca.* 120 applications yr^{-1}) were supplied automatically when air temperature > 0 °C and wind speed < 5 m s^{-1} (Sheppard et al., 2004).

2.3. Sphagnum *pore water*

Mini rhizon suction samplers (Rhizon MOM, Eijkelkamp Agrisearch Equipment, Wageningen, The Netherlands) attached to a 20 mL plastic syringe were used to collect pore water samples from the open *Sphagnum* moss layer. The sampler was inserted into the *Sphagnum* layer (5cm depth) to evaluate how active the living part of *Sphagnum* was at removing nutrients. In August 2013, one collector was placed in each plot. Aluminium foil wrapped the syringe and connectors attached to the rhizon samplers to avoid light penetration into collected pore water. The location of the collector for *Sphagnum* pore water was fixed until October 2013. Collection was made weekly during the period from August 2013 to October 2013.

The collected pore water samples were immediately transported back to the nearby laboratory and were filtered through a 0.45 μm membrane filter (Puradisc^{TM}, Whatman Inc., NJ, USA). The filtered samples were stored in the dark at 4°C until chemical analysis. NO_3^- and NH_4^+ were analysed by ion chromatography (CH-9101, Metrohm, Herisau, Swizerland) and Ammonia Flow Injection Analyser (AMFIA, ECN; Wyers *et al.* 1993), respectively. Dissolved inorganic N (DIN) concentrations were calculated as the sum of NO_3^- and NH_4^+.

2.4. Tissue nutrient concentrations of Sphagnum *moss*

Sphagnum vegetation samples were collected at the beginning of December 2013 to diagnose the nutrient condition of *Sphagnum* treated over 11 years. A few shoots per plot were collected from where the pore water was sampled and combined to give one composite sample per plot. The litter on the collected *Sphagnum* was thoroughly removed using tweezers. The samples were separated into capitula (0-1 cm) and stem (>1 cm) fractions and were dried at 70 ºC for 72 h. Total N content in capitula and stem of *Sphagnum* were measured using a CN analyzer (CN corder MT-700, Yanaco Co., Ltd., Tokyo, Japan). To analyze total P, the dried samples were burned at 550 ºC for 2 hr and then digested using potassium peroxodisulfate ($K_2S_2O_8$). Total P concentration in digested solution was measured using molybdenum blue (ascorbic acid) spectrophotometric method (UV mini-1240, Shimadzu, Kyoto, Japan). To ensure accuracy within 5% of known N and P concentrations, standard reference material (NIST 1515 Apple Leaves, National Institute of Standards and Technology, Maryland, USA) was analyzed along with *Sphagnum* samples.

2.5. Calculation and statistical analysis

Student's t-test was used to assess differences in tissue nutrient and pore water quality of the *Sphagnum* layer between treatments with and without P and K. The

Mann–Kendall test was performed to evaluate annual trends in the capitulum N concentrations. All statistical analyses were carried out using SPSS 22.0J (SPSS Japan Inc.).

3. Results and Discussion

3.1. Alleviation effects of long-term P and K addition on N status of Sphagnum

Previous studies have indicated that P and K addition alleviates the adverse effects of short-term N addition on *Sphagnum* physiological status, with positive effects on assimilation N (Limpens et al., 2004), growth (Limpens et al., 2004; Carfrae et al., 2007; Lund et al., 2009; Kivimäki, 2011; Fritz et al., 2012) and cover (Pilkington et al., 2007). In an earlier study at the same site Carfrae et al. (2007) reported that P and K additions reduced N accumulation (decrease in tissue N concentration) for N_{red} plots after only one year of treatment. The reduction in N accumulation (decrease in tissue N concentration) of *Sphagnum* capitula (22% decrease) and stems (20% decrease) can also be seen over 4 and 5 years treatments (Fig. 2c). Kivimäki, (2011) also showed that adding P and K increased shoot extension (16-27 mm) compared to ‘N only’ treatments (13-17 mm) after 5 years of treatments at this study site.

This long-term study, however, showed that P and K additions will not offset the detrimental impacts of long-term high N deposition. P and K additions did not affect capitulum N concentrations for reduced N treatments (*P*=0.95, Fig. 1a) but tended to cause lower stem N concentrations (*P*=0.066, Fig. 1b). The N saturation of *Sphagnum* was caused by adding wet deposition of 56 kg N ha^{-1} yr^{-1} of reduced N over 11 years (Chiwa et al., 2016b). The P and K additions over 11 years did increase capitulum and stem P concentrations (Fig. 1cd) causing subsequently lower N:P ratios (Fig. 1ef) suggesting that the P dose exceeded growth requirements. The lower stem N concentrations with P and K (Fig. 1b) indicate some growth enhancement was induced, providing some amelioration from the excess N. However, capitulum N concentrations remained consistently high for N_{red} 56PK over 11 years, similar to those for N_{red} 56 (Fig. 1a, Fig. 2c), indicating that P and K addition only partially alleviate N saturation of *Sphagnum* exposed to N addition over 11 years.

The results suggest that in the short term, the high dose does not saturate *Sphagnum*, thereby allowing the effect of P and K, probably via growth enhancement, to lower N concentrations. In support of this view, when stem N concentrations of *Sphagnum* for N_{red} 56 over the first 5 years remained low, capitulum N concentration was reduced by P and K addition (Fig. 2c). Addition of P and K has a different effect over time on the N content of stem and capitulum, implying differences in metabolism and storage of nutrients and/or internal transport processes in response to continuing nutrient stresses.

For oxidized N plots, P and K additions did not affect either capitulum or stem N concentrations (Fig. 1ab). In addition, although the alleviation effects by P and K addition were found for short-term addition of reduced N (Fig. 2c), the effect was not found for oxidized N even for short-term as well as long-term manipulation. Stem N concentration of *Sphagnum* for N_{ox} 56 was not affected for oxidized N even over the long-term (Fig. 2b). These results indicate that the alleviation effects by P and K addition for oxidized N are smaller than for reduced N. The reason remains unclear, but could be related to the difference of growth response of *Sphagnum* to P and K addition. *Sphagnum* production exposed to N_{red} 56 over 5 years (82 g m^{-2} yr^{-1}) increased to 198 g m^{-2} yr^{-1} (N_{red} 56 PK), whereas the increase in the productivity of *Sphagnum* exposed to N_{ox} 56 over 5 years (73 g m^{-2} yr^{-1}) was smaller (86 g m^{-2} yr^{-1} for N_{ox} 56PK) (Kivimäki, 2011).

3.2. Alleviation effects of long-term P and K addition on N assimilation of Sphagnum

Limpens et al. (2004) has shown that P addition (3 kg P ha^{-1} yr^{-1}) improved N assimilation capacity of *Sphagnum* exposed to N (40 kg N ha^{-1} yr^{-1}), over 4 years.

However, adding N_{red} significantly increased DIN concentrations in pore water from within the *Sphagnum* layer cf controls (Fig. 3) but adding P and K made no difference (*P*=0.29) and average DIN concentrations for N_{red} 56 +/- PK remained above 100 μmol l^{-1} (Fig. 3). Thus adding P and K hardly influenced mineral N retention by alleviating N saturation of *Sphagnum* in this study. The difference could be caused by the difference of manipulation duration. These two studies suggest any amelioration effect of P and K on N retention changes over time, probably depending on the stage of N saturation.

In contrast to N_{red}, there was a significant difference between DIN (*P*=0.034) and NO_3^- (*P*=0.019) concentrations for N_{ox} 56 and N_{ox} 56PK. (Fig. 3). Thus, the alleviation effects of P and K addition on N assimilation of *Sphagnum* were observed for oxidized N, which could be related to the stage of N saturation of *Sphagnum*. Chiwa et al. (2016b) found that the effect of oxidized N on advancing N saturation was lower than that of reduced N and that the stage of N saturation of *Sphagnum* exposed to N_{ox} 56 over 11 years had not advanced compared to that for N_{red} 56. NO_3^- uptake by *Sphagnum* caused DON leaching from *Sphagnum* that enables *Sphagnum* to delay N saturation of *Sphagnum* (Chiwa et al., 2016b).

4. Conclusions

This study concludes that long-term additions of P and K have no major ameliorating effects on a *Sphagnum* moss subjected to continuous high N inputs. There were different minor effects depending on the form of N, with some lowering of N concentrations for reduced N, but for oxidized N the chemical effects were small even though the detrimental effects on *Sphagnum* cover were massive. These results show that P and K additions will not offset the N saturation of *Sphagnum*, and in some cases, where N deposition is predominantly in the oxidized form, may exacerbate any effects of N alone.

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Figure Captions

Fig. 1. *Sphagnum* tissue N concentration of a) capitulum and b) stem; tissue P concentration of c) capitulum and d) stem, and N:P ratio of e) capitulum and f) stem without P and K (white bar) and with P and K (grey bar). Bars represent standard error ($n = 4$). Asterisk indicates significant differences at $P<0.05$. Background N deposition is *ca.* 8 kg N ha^{-1} yr^{-1} (Leith et al., 2004; Sheppard et al., 2004).

Fig. 2. Annual trends in capitulum and stem N concentrations of *Sphagnum* on Whim bog in south Scotland. N concentration 0, 2, 4, 5 and 7 years after N manipulation started were taken from Sheppard et al. (2004), Carfrae et al. (2007), Phuyal et al. (2008), Kivimäki (2011), and Manninen et al. (2011) respectively.

Fig. 3. *Sphagnum* pore water concentrations of dissolved inorganic nitrogen (DIN, NO_3^- + NH_4^+). Bars represent standard error ($n = 4$). Asterisk indicates significant differences at $P<0.05$.

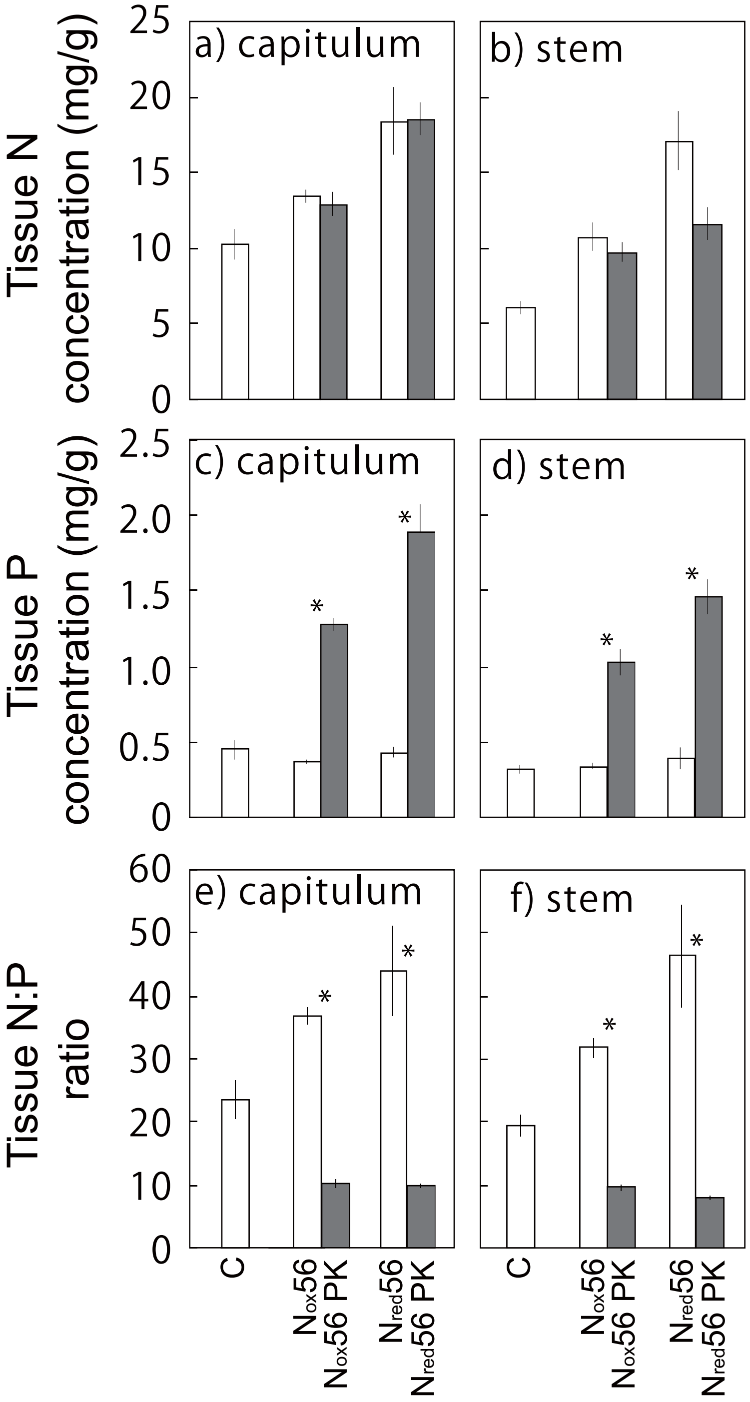

Fig. 1
Chiwa et al.

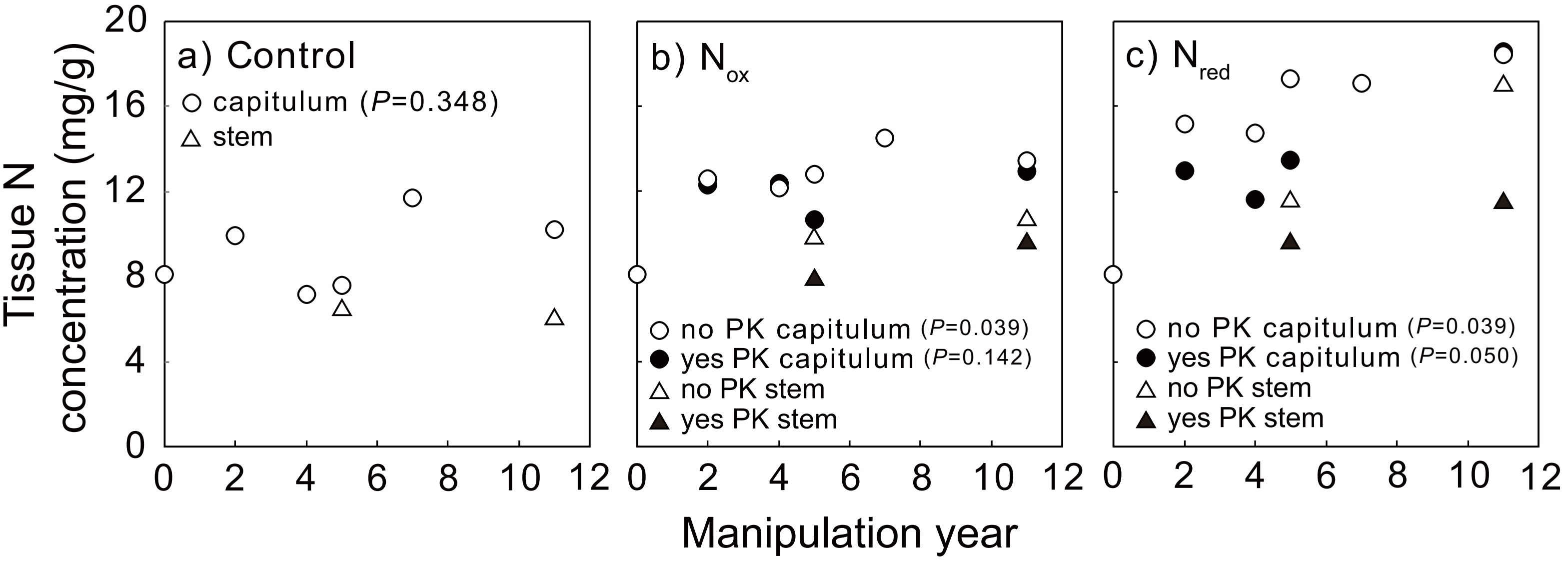

Fig. 2
Chiwa et al.

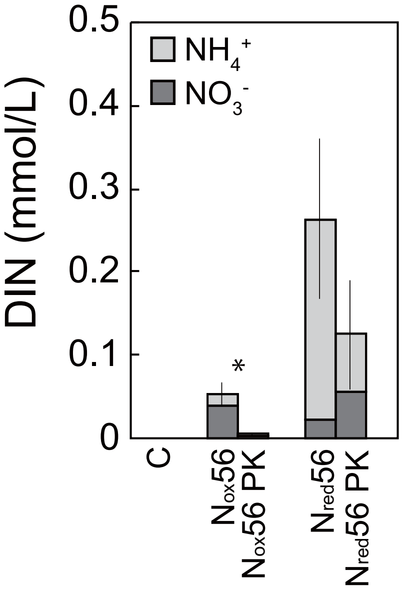

Fig. 3
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