Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils

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ABSTRACT

Drying and rewetting of soils triggers a cascade of physical, chemical, and biological processes; understanding these responses to varying moisture levels becomes increasingly important in the context of changing precipitation patterns. When soils dry and water content decreases, diffusion is limited and substrates can accumulate. Upon rewetting, these substrates are mobilized and can energize hot moments of intense biogeochemical cycling, leading to pulses of trace gas emissions. Until recently, it was difficult to follow the rewetting dynamics of nutrient cycling in the field without physically disturbing the soil. Here

we present a study that combines real-time trace gas measurements with high-resolution measurements of diffusive nutrient fluxes in intact soils. Our goal was to distinguish the contribution of different inorganic and organic nitrogen (N) forms to the rewetting substrate flush and the production of nitric oxide (NO) and nitrous oxide (N_2O). Diffusive flux of N-bearing substrates (NO_2^-, NO_3^-, NH_4^+ and amino acids) was determined *in situ* in hourly resolution using a microdialysis approach. We conducted an irrigation experiment in a semi-arid California grassland at the end of the dry season, and followed soil N flux and N trace gas emissions over the course of 30 h post-wetting. Upon rewetting, both inorganic and organic N diffused through the soil, with inorganic N contributing most to the rewetting N flush. Emissions of NO and N_2O rapidly increased and remained elevated for the duration of our measurements, whereas diffusive soil N flux was characterized by large temporal variation. Immediately after rewetting, NO_3^- contributed 80 % to the total diffusive N flux but was consumed rapidly, possibly due to fast microbial uptake or denitrification. Ammonium flux contributed only ~10 % to the initial diffusive N flux, but it dominated total N diffusion 27 h post-wetting, coinciding with peak N-gas emissions. This suggests that at this time point, most of the N trace gases were produced via biological nitrification. Nitrite contributed only 1 % to total N diffusion and did not show a clear temporal pattern. Amino acids contributed roughly as much as NH_4^+ to the initial diffusive N flux, but the organic N pulse was short-lived, indicating that organic N did not contribute substantially to N-gas formation shortly after rewetting at our study site. In conclusion, our results support the hypothesis that in semi-arid environments N-bearing substrates concentrate during dry periods and, upon rewetting, can lead to pulses of NO and N_2O when they react chemically or are transformed by microorganisms.

1. Introduction

Periods of drought are common in most terrestrial ecosystems; hence the influence of drying and rewetting on soil processes has been central in ecosystem research. This becomes even more important with projected increases in extreme weather events (IPCC, 2014). Rewetting triggers a cascade of responses in soil physical and chemical processes (Homyak et al., 2016) and shifts in microbial physiology (Placella and Firestone, 2013). For nitrogen (N), drying concentrates N-containing substrates in hydrologically disconnected microsites which, upon rewetting, can produce both nitric oxide (NO), an air pollutant, and nitrous oxide (N_2O), a powerful greenhouse gas (Galbally et al., 2008). When soils rewet, it is thought that a flush of inorganic N [ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-)] governs both

58 the abiotic and biotic transformations that produce N emission pulses. In drylands it has been suggested
59 that the most important processes of NO production following a rewetting event are i) flushing and rapid
60 abiotic chemo-denitrification of NO_2^- and ii) biotic nitrification of NH_4^+ (Davidson, 1992, Homyak et al.,
61 2016). Nitrous oxide is assumed to be mainly produced via nitrification in dry soils (Davidson, 1992;
62 Beare et al., 2009), but after rewetting when high microbial activity leads to O_2 depletion, denitrification of
63 nitrate (NO_3^-) or NO_2^- can also contribute to N_2O emissions (Venterea and Rolston, 2000; Ruser et al.,
64 2006; Galbally et al., 2008). While the processes that lead to N-gas formation have largely been identified
65 (Butterbach-Bahl et al., 2013; Pilegaard, 2013), we know little about the temporal dynamics of inorganic N
66 accumulation and flushing upon rewetting, and how these substrates may synchronize to sustain trace gas
67 emission pulses. Even less attention has been given to the dynamics of organic N and whether it
68 contributes to these emission pulses.
69 Determining the rewetting dynamics of N compounds has been challenging because it is difficult
70 to monitor minute- to hour-scale changes in N supply in intact soil. Studies have mostly relied on
71 destructive sampling, but disturbances during soil collection and analysis can alter microbial processes
72 (Dumont et al., 2006; Lee et al., 2007) and N concentrations (Rousk and Jones, 2010; Warren and Taranto,
73 2010; Inselsbacher, 2014). For instance, destructive sampling may overestimate N availability because
74 bulk soil extractions may release protected N in organo-mineral complexes that had not been available for
75 microbial uptake (Van Gestel et al., 1991; Fierer and Schimel, 2003). The N that actually diffuses to
76 microbes and reactive microsites upon rewetting is not well quantified, leading us to ask: What are the
77 dominant forms of inorganic and organic N that are bioavailable during a rewetting pulse? How do the
78 concentrations of these substrates vary across time? And does peak diffusive N flux coincide with peak NO
79 and N_2O emission pulses?
80 We answered these questions in intact soils by using microdialysis to capture N diffusion
81 dynamics (Inselsbacher et al., 2011) coupled with measurements of NO and N_2O emissions. Similarly to
82 microorganisms or plant roots, microdialysis probes collect substrates diffusing through the soil solution
83 (Ginige et al., 2004), allowing us to determine diffusive N fluxes upon rewetting. We hypothesized that: i)
84 rewetting would cause a NO_2^- flush coinciding with rapid emissions of N gases, ii) substrates consumed by
85 biological processes would decrease after the rewetting pulse, and iii) available N-bearing organic
86 substrates would decrease after wetting.
87

2. Materials and Methods

The study site was located in a seasonally-dry oak savanna in the University of California Sedgwick Reserve (N 34.7120, W 120.0388; 370 m asl). Vegetation is dominated by Mediterranean annual grasses (*Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*). The soil is a thermic Pachic Haploxeroll (pH 6.9, 2.2% C, 0.21% N, 1.2 g cm^{-3} BD, upper 10 cm) on flat slopes (Blankinship et al., 2016). The mean annual temperature is 16.8 °C. Annual precipitation averages 380 mm, with most falling between November and April.

In early November 2015, before onset of the winter growing season, we irrigated a soil plot (2 m x 1 m) with 30 L (corresponding to 15 mm rainfall) of local well water (0.003 mg NH_4^+-N L^{-1}, 1.6 mg NO_3^--N L^{-1}, 0.4 mg DON L^{-1}). Fluxes of NO and N_2O were determined by chamber methodology (Davidson et al., 1991) 1 h before and every 1-4 hours post-wetting over the course of 30 h, with a pause between 11 h and 24 h post-wetting. One portable dynamic chamber (30.5 cm diameter, 10 cm height) was connected to a chemiluminescent NO analyzer (Scintrex LMA-3, Canada) and an Off-axis ICOS N_2O laser analyzer (Los Gatos Research, CA, USA). During gas flux measurements, the chamber was consecutively placed in each corner of the experimental plot, with at least 15 cm distance to the plot edges. Closure time was 5 min for each location, and between measurements the chamber was vented until concentrations returned to ambient levels (~60 s). To ensure airtight sealing and to dampen pressure fluctuations inside the chamber, the chamber was equipped with a 20 cm long polyethylene skirt at its base (Parkin and Venterea, 2010). Fluxes were calculated based on the rate of change in gas concentration inside the chamber after correcting for air temperature and air pressure (Homyak et al., 2016).

Microdialysis probe calibration and soil sampling was performed according to Inselsbacher et al. (2011). Four flow-through polyarylethersulphone probes (CMA 20, 10 mm long, 500 µm diameter, 20 kDa molecular weight cut-off; CMA Microdialysis AB, Sweden) were installed vertically down to 2.5 cm soil depth after creating a pilot hole with a cannula. The probes were positioned in a square at the center of the plot, with 50 cm distance between each probe, and left at the same location for the duration of our measurements. High-purity deionized water (MilliQ) was pumped through the system using a syringe infusion pump (CMA 400, flow rate 5 µl min^{-1}), and samples were collected hourly in a refrigerated microfraction collector (6 °C; CMA 470), with a pause between 11 and 24 h after rewetting. Every 3 h, samples were taken out of the microfraction collector and stored in a cool box on ice until frozen (-20 °C) within 24 h of collection. Diffusive N fluxes from the soil solution were calculated based on membrane

surface area and time and expressed as μg N cm^{-2} h^{-1} (Inselsbacher and Näsholm, 2012). We also sampled the upper 5 cm of soil using a 4-cm diameter corer within 20 cm of the microdialysis probes prior to wetting and 1, 8, 24 and 30 h post-wetting. In the lab, soil was extracted in either 0.5 M K_2SO_4 or MilliQ water. Microdialysis and soil extract samples were analyzed colorimetrically for NO_2^- (Homyak et al., 2015), NO_3^- and NH_4^+ on a plate reader (Hood-Nowotny et al., 2010). Seventeen amino acids were analyzed by reverse-phase liquid chromatography (see Supplementary methods for details) on a UPLC system equipped with a fluorescence detector (Waters Corp., MA, USA). Statistical analysis was done with R 3.3.2 (www.r-project.org).

3. Results

Nitric oxide emissions increased 25-fold upon rewetting, from 0.29 ± 0.05 ng N m^{-2} s^{-1} pre-wetting to 7.47 ± 1.26 ng N m^{-2} s^{-1} 1 h post-wetting (Figure 1a), and they continued to increase until reaching a peak at 41.6 ± 2.0 ng N m^{-2} s^{-1} 8 h post-wetting. Following this peak, fluxes declined to approximately 25 ng N m^{-2} s^{-1} drifting to values below 20 ng N m^{-2} s^{-1} by the end of our measurements. Nitrous oxide emissions also increased immediately after rewetting, from -0.04 ± 0.10 ng N m^{-2} s^{-1} pre-wetting to 5.57 ± 1.27 ng N m^{-2} s^{-1} within 1 h post-wetting (Figure 1b). Compared to NO, the increase in N_2O emissions was slower and highest after 27 h (12.95 ± 6.03 ng N m^{-2} s^{-1}).

Microdialysis requires moist conditions for substrates to diffuse into the probes; therefore, we were unable to determine pre-wetting diffusive fluxes. Nitrate accounted for ~80 % of the total diffusive N flux immediately after the rewetting pulse (Figure 2a); fluxes were highest during the first 2 h post-wetting (0.70 ± 0.47 μg N cm^{-2} h^{-1}), but decreased rapidly and remained low for the duration of our measurements (Figure 3a). Nitrite amounted to only 1 % of the total diffusive N flux (Figure 2a). During the first 10 h after rewetting, diffusive NO_2^- flux averaged 0.03 ± 0.004 μg N cm^{-2} h^{-1} (Figure 3b), but decreased by ~50 % by the second day. Ammonium represented 9 % of the total diffusive N flux immediately after rewetting (Figure 2a). Similarly to NO_3^-, we observed an initial flush of NH_4^+ during the first 2 h post-wetting, when fluxes averaged 0.07 ± 0.04 μg N cm^{-2} h^{-1}, but the pulse was short-lived (Figure 3c). After ~27 h, NH_4^+ diffusive fluxes increased to a high of 3.56 ± 2.84 μg N cm^{-2} h^{-1}, the highest diffusive N flux we measured. Initially, the amino acid flux was in the same range as initial NH_4^+ and contributed 10 % to total diffusive N flux (Figure 2a); flux was highest in the first 2 h post-wetting (0.07 ± 0.03 μg N cm^{-2}

147 h^{-1}) but then rapidly dropped by ~75 % to around 0.02 ± 0.01 μg N cm^{-2} h^{-1}, where flux remained into the
148 second day of measurement (Figure 3d).

149 The distribution of inorganic N species in water-extracted soils was similar to that of
150 microdialysis, with NO_3^- accounting for 63 % of the total N pool, 18% NO_2^-, 7% NH_4^+, and 12% amino acids
151 (Figure 2b). In K_2SO_4 extracts, NH_4^+ concentrations where higher compared to water extracts (*t*-test, $P <$
152 0.01); NH_4^+ made up the largest fraction of the exchangeable N pool (53 %), compared to 7 % NO_2^-, 28 %
153 NO_3^-, and 12 % amino acids (Figure 2c). In contrast to microdialysis, bulk soil N concentrations did not
154 change significantly between pre- and post-wetting conditions (one-way ANOVA, $P > 0.05$).
155

156 ## 4. Discussion

157 During dry periods, mineral and organic N substrates are hypothesized to accumulate in soil
158 because of (i) decreased plant N uptake, and (ii) because soil microsites where decomposition and N
159 mineralization take place become hydrologically disconnected from microsites of microbial N
160 immobilization (Parker and Schimel, 2011; Homyak et al., 2016). Our results show that N-bearing
161 substrates were rapidly mobilized upon rewetting, and this mobilization coincided with rapid increases in
162 N trace gas emission pulses.

163 Both NO and N_2O emissions increased rapidly within the first hour after rewetting, and emissions
164 continued over the next 30 h. Theory suggests that in arid and semi-arid ecosystems when soils are at low
165 to intermediate water contents NO and N_2O are primarily produced via nitrification (Davidson, 1992).
166 However, rapid chemical reactions involving NO_2^- (chemodenitrification) contribute to these emissions
167 (Medinets et al., 2015; Heil et al., 2016). At our study site, nitrification potentials increase during the dry
168 season (Parker and Schimel, 2011), and NO_2^- chemodenitrification upon rewetting is responsible for
169 generating rapid NO emission pulses (Homyak et al., 2016). Consistent with this understanding, there was
170 ongoing diffusive NO_2^- flux throughout our experiment, which could have stimulated chemodenitrification,
171 especially upon rewetting. Furthermore, diffusive NH_4^+ flux increased 27 h after the rewetting pulse,
172 which coincided with the period of highest N gas emission. This suggests that as microbes recover from
173 drought-induced stress, increasing mineralization and NH_4^+ supply may contribute to N gas emission
174 pulses via nitrification.

Immediately after rewetting, NO_3^- made up the majority of the initial diffusive N flux but then rapidly disappeared, suggesting it was immobilized or denitrified. In drylands, denitrification is usually low because soils are well-aerated but denitrification is anaerobic (Venterea and Rolston, 2000; Galbally et al., 2008). Although we did not measure nitrification and denitrification rates, Parker and Schimel (2011) found that denitrifying enzyme activity increased during the dry season at our study site, suggesting denitrification may be important immediately post-wetting. Indeed, N_2O emissions increased within minutes post-wetting to a maximum of 13 ng N m^{-2} s^{-1} 27 h after irrigation. Moreover, we found a negative correlation between NO_3^- diffusion and N_2O emissions (r = -0.89, p < 0.001, Supplementary. Table 1), perhaps suggesting that NO_3^- was in fact reduced to N_2O. It has been suggested that bursts of microbial respiration following a rewetting pulse can rapidly deplete soil oxygen levels, allowing denitrification to occur in anoxic soil microsites and leading to substantial N_2O emissions in drylands (Hu et al., 2017). Therefore, it seems likely that the increase in soil moisture together with the initial NO_3^- flush during the rewetting pulse created conditions favorable for denitrification, which could have contributed to the observed N gas emissions and the drawdown of NO_3^- in soil.

Another explanation for the decrease in NO_3^- diffusion could be the formation of a diffusional depletion zones around the microdialysis membranes, which would occur if diffusion through the microdialysis membrane decreased local N faster than it was resupplied from the surrounding soil (Inselsbacher et al., 2011). However, this seems unlikely for several reasons: i) depletion zones are more likely to form for cations like NH_4^+ that bind to negatively charged surfaces like clay minerals and soil organic matter and are thus less mobile in soil, but NH_4^+ diffusion was high at the end of our measurements after 26 h even though the microdialysis membranes were kept at the same spot; ii) small anions like NO_2^- and NO_3^- or neutral and acidic amino acids can move easily through the soil solution, which makes the formation of diffusional depletion zones less likely; and iii) depletion zone formation is less likely if the production rates of the respective compounds are high and constantly provide a resupply of these molecules, keeping the concentration gradient intact. Given that NO and N_2O emissions were high after the rewetting, it seems plausible that also the rate of NO_2^- and NO_3^- production were high.

It should be noted that the irrigation water used in the present study contained 1.6 mg NO_3^--N L^{-1}, corresponding to 0.24 kg N ha^{-1}. This is similar to NO_3^- concentrations in rainwater for the southwestern US (0.5 to 1.5 kg NO_3^--N ha^{-1}; Holland et al., 2005) and within the range of expected atmospheric N deposition for our site (5-7 kg N ha^{-1} yr^{-1}; Fenn et al., 2010). Based on a soil porosity of 54 %, we expected

205 the irrigation water to infiltrate to a depth of at least 3 cm, wetting 66 kg of soil. Under these conditions,
206 and assuming steady state, irrigating soils would have raised the NO_3^- content of the soil (4-6 μg NO_3^--N g^{-1}
207 dw) by only 0.7 μg NO_3^--N g^{-1} dw, or by at most 18 %. Therefore, well water addition, alone, is unlikely to
208 explain the NO_3^- patterns detected using microdialysis.
209 Considering the role of organic N, amino acids contributed about as much to initial N flux as NH_4^+,
210 but the amino acid flush was short-lived. The dominant amino acids were methionine, valine and tyrosine
211 (Supplementary Figures S1 and S2), but we found no indication of known microbial osmolytes like proline,
212 which may be synthesized by microorganisms experiencing drought stress (Killham and Firestone, 1984;
213 Csonka, 1989). Our findings are consistent with previous studies that have reported no *in-situ* osmolyte
214 accumulation in drying soils (Boot et al., 2013; Göransson et al., 2013; Kakumanu et al., 2013). It has been
215 suggested, however, that even if osmolytes are produced by drought-stressed microorganisms, they may
216 be less likely to be disposed into the soil solution after rewetting; instead, osmolytes may be mineralized
217 intracellularly since they represent a valuable C and N source (Warren, 2014). In the context of N trace gas
218 formation, organic N has been proposed to directly contribute to N_2O formation via heterotrophic
219 nitrification (Müller et al., 2014). However, because amino acids were unavailable beyond the first 2 h
220 post-wetting, they are unlikely to directly account for a significant fraction of N_2O production at our site.
221 In contrast to microdialysis results, soil extracts did not show differences in N availability before
222 and after rewetting, likely because bulk extractions integrate N pools that turn over at different rates (i.e.,
223 slow and fast cycling pools). This highlights the potential of the microdialysis approach, which allowed us
224 to measure the dynamics of bioavailable pools at high temporal resolution, which is required to capture
225 short-term changes in N availability during pulsed events (Homyak et al., 2017). The present study
226 highlights that N fluxes in soil can change very rapidly—with microdialysis we have a tool to catch this
227 dynamic. Furthermore, it allows us to determine soil N fluxes *in situ*, without the workaround of taking soil
228 samples and conducting lab incubations; this provides us with a more realistic picture of what is really
229 going on at the microbial scale in an intact ecosystem. In conclusion, our study showed rapid soil N
230 dynamics following a rewetting pulse that include an immediate draw-down of NO_3^- and amino acids
231 followed by a stimulation of ammonification that began 24 hours following rewetting. These shifts in the
232 availability of different N-forms corresponded to shifts in the fluxes of NO and N_2O. Observations of both
233 soil N diffusion and trace gas emissions were enabled by combination of new microdialysis and trace-gas
234 measurements that allowed evaluation of short-term dynamics of N transformations. In this semi-arid

grassland, microbial processes controlled emissions of N gases both by generating substrates that concentrate in dry soils and react chemically upon rewetting (i.e., NO_2^-), and by generating substrates that stimulate biological production of N gases as microbes recover from drought stress (NO_3^-, NH_4^+).

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FIGURES:

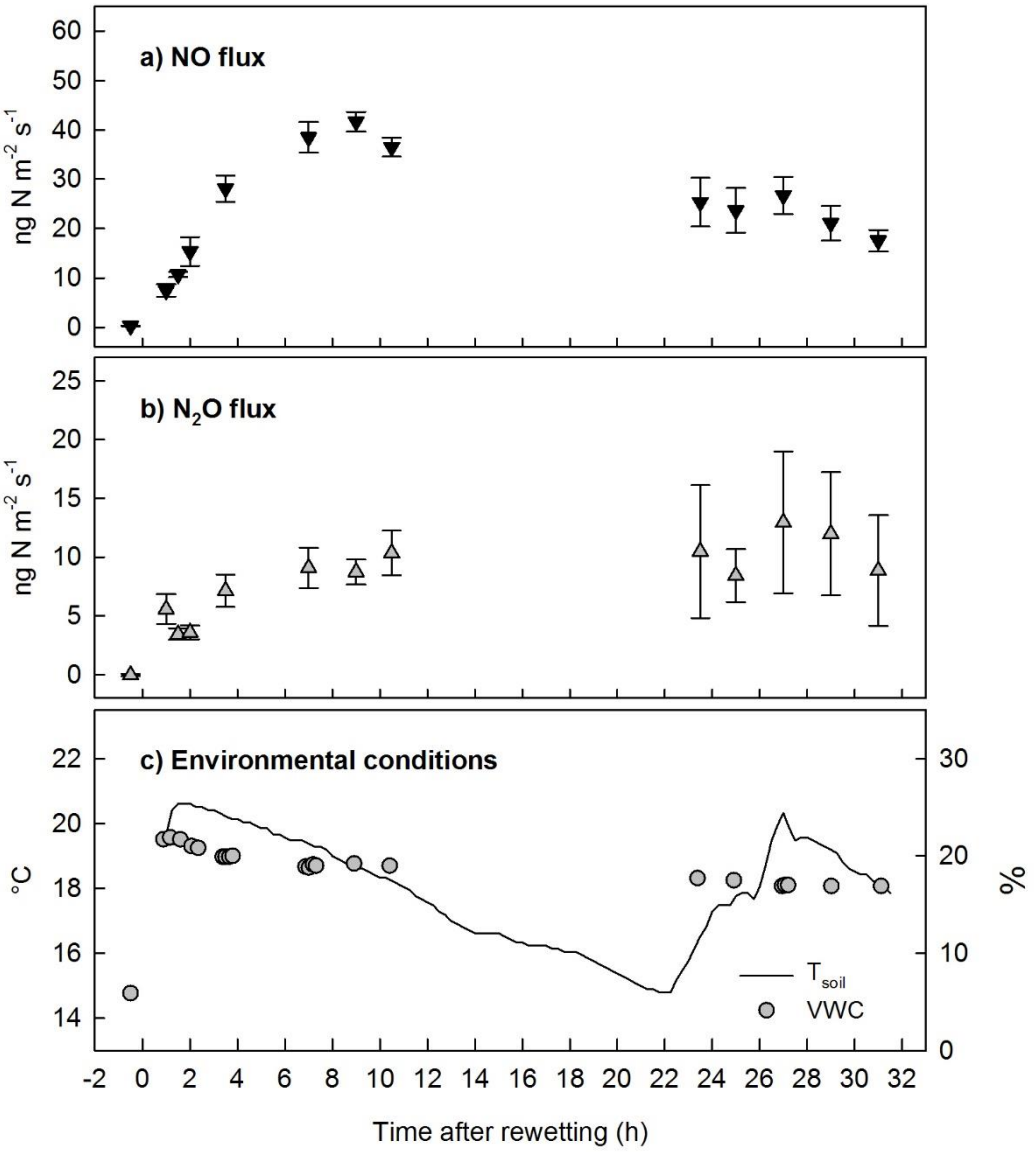

Figure 1: Fluxes of a) nitric oxide (NO) and b) nitrous oxide (N_2O), and c) soil temperature (T_{soil}) and volumetric water content (VWC). The first time point was measured in dry soil; all consecutive times were measured after irrigating soils with 15 mm of water. Data are average ± SE (n = 4).

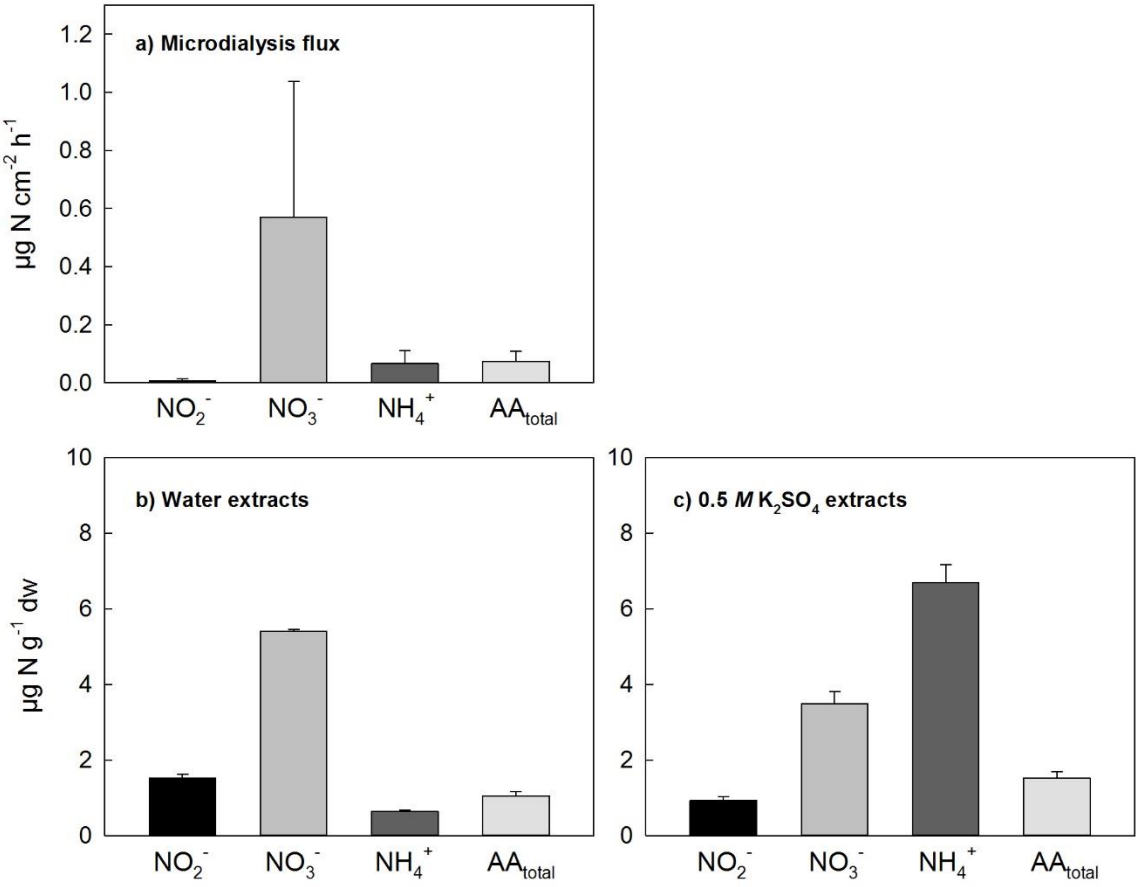

Figure 2: Contribution of inorganic and organic N to a) diffusive N flux measured by microdialysis at the first sampling time point 1 h after rewetting (upper panel, average ± SE, n = 4), and concentrations determined by extracting soils with b) MilliQ water or c) 0.5 *M* K_2SO_4 (lower panel, average ± SE, n = 3).

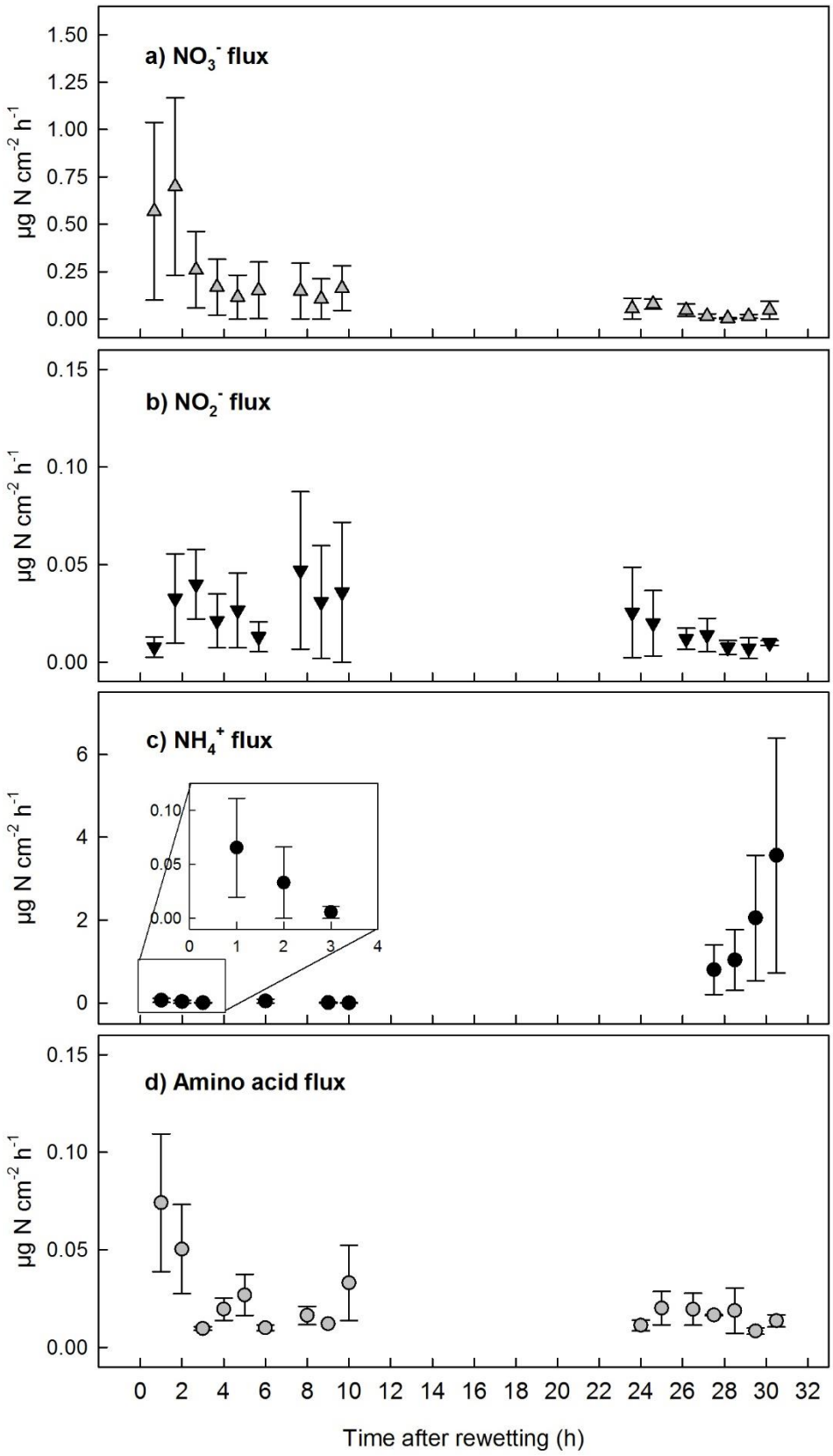

Figure 3: Diffusive fluxes of a) nitrate (NO_3^-), b) nitrite (NO_2^-), c) ammonium (NH_4^+) and d) sum of 17 amino acids determined *in situ* with microdialysis over the course of 30 h after irrigating soils with 15 mm of water (average ± SE, n = 4).

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