



# Soil biogeochemical responses to multiple co-occurring forms of human-induced environmental change

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## Abstract

Human activities cause a multitude of environmental issues, including increased temperatures and altered precipitation patterns associated with climate change, air pollution, and other impacts of urbanization. One area highly affected by these issues is the Sonoran Desert, specifically the Phoenix metropolitan area where urbanization is among the most rapid in the United States. Most studies investigate these multiple environmental change factors independently or sometimes in pairs, but rarely all together as co-occurring forms of change. We examined how the simultaneous manipulation of increasing temperatures, altered precipitation patterns, nitrogen deposition, and urbanization influenced soil respiration and mineral N pools in the Sonoran Desert. Soil was collected from urban and exurban sites, from both nitrogen-fertilized and control plots. To simulate projected climate change, the soils were incubated in microcosm at the annual average Phoenix temperature as well as a 2 °C increase under a factorial precipitation treatment of decreased frequency and increased pulse size. Our results show that C and N dynamics were altered by all four forms of environmental change. However, the dominance of significant 3- and 4-way interactions among the four environmental factors for both respiration and mineral N pools demonstrates that the impact of any given form of environmental change will depend on the levels of the other environmental factors. In other words, the cumulative effect of altered precipitation, fertilization, temperature, and urbanization on soil biogeochemical processes is not necessarily predictable from their individual impact.

**Keywords** N deposition · Climate change · Altered precipitation · Urbanization · Soil respiration

## Introduction

The carbon (C) cycle of terrestrial ecosystems is an important component of understanding climate change. The soil is a large component of the terrestrial C sink that helps slow the rise of atmospheric CO<sub>2</sub> and offset C emissions (Ballantyne et al. 2012; Wang et al. 2020). One flux of C out of this soil storage is soil respiration, the flux of C from the soil to the atmosphere, and is responsible for 60–90% of total ecosystem respiration (sensu Wang et al. 2014a). Due to the large impact that soil respiration has on an ecosystem, it is important to understand what drives the soil microbial

activity that accounts for a large portion of soil respiration. There is a particular lack of data on desert soils, which have low productivity and respiration rates (sensu Cable et al. 2011) yet store a lot of C (Wang et al. 2010). As such a large C sink, the soil interacts with other organisms in the ecosystem to sustain life. This makes it a valuable part of the environment and drives the need to understand the processes that control soil activity. Water availability and temperature are the most important controlling factors in the Sonoran Desert for soil respiration, which increases after rainfall events and decreases with higher temperatures (Bilderback et al. 2021). Since respiration is influenced by temperature and moisture, climate change will most certainly impact this soil process.

Rainfall pulse size and frequency are expected to change in the form of more intense drought periods and an increased frequency of large rainfall events, as a result of climate change in the Sonoran Desert (Cayan et al. 2010; Cook and Seager 2013; Georgescu et al. 2021). These changes in precipitation patterns are a result of altered circulation patterns that are caused by changes in temperature, which

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are expected to continue to rise (IPCC 2021). The duration of the rainfall pulse, as well as the pulse size, will determine the extent to which the soil is impacted and for how long. Small rainfall events will only reach the upper part of the soil, where soil microorganisms are concentrated, whereas larger events will reach a deeper portion of the soil (Schwinning and Sala 2004). During rainfall events, soil nutrients and water needed for productivity and respiration are in abundance, and then become scarce during dry periods (Schwinning and Sala 2004). In a study done in the Chihuahuan Desert, soil respiration was higher when rainfall pulses were large rather than when the same amount of rain was distributed in smaller rainfall events (Vargas et al. 2012). In a similar study conducted in the Sonoran Desert, experimental pulses simulating 1 cm of rainfall significantly increased fluxes of CO<sub>2</sub>, N<sub>2</sub>O, and NO during the dry season, but resulted in only slight increases or even decreases of CO<sub>2</sub>, N<sub>2</sub>O, and NO (Harms and Grimm 2012). This suggests that arid ecosystems with less-frequent pulse events will cause soils to respire more when they are subjected to rainfall events, and therefore, intensifying aridity could result in increased soil respiration. However, a few studies have confirmed this. It still largely remains unclear how intensifying aridity (Seager et al. 2007) will affect microbially mediated soil processes, such as respiration and nutrient availability, in arid ecosystems. Previous research suggests that an increase in precipitation could stimulate soil CO<sub>2</sub> emissions, but it is uncertain by how much (Guan et al. 2021; Tan et al. 2021; Zhao et al. 2021). Consequently, decreased precipitation could also cause a decrease in soil respiration (Li et al. 2020; Wang et al. 2021). Further, variability in the size and timing of pulse events can have complex effects on aridland C dynamics (Griffin-Nolan et al. 2021). Fewer studies investigate consequences for soil nutrient availability, but there is evidence that drought and altered timing of precipitation can increase mineral N and P availability in drylands (Holguin et al. 2022; Wu et al. 2022).

Beyond precipitation, some studies from semi-arid environments suggest that increased temperatures result in increased soil respiration (Fang et al. 2017; Peri et al. 2015), at least up to a point (Cable et al. 2011). Many studies have investigated the responses of plants to temperature change; however, few have explored the responses of organisms below ground in arid ecosystems. Soil microbes respond more quickly to changes in temperature and moisture than do plant communities, which results in sudden changes in soil respiration, though hot desert communities may be less temperature sensitive (Cable et al. 2011). For these reasons, it is important to understand how soil microbial activity in the Sonoran Desert will be impacted as a result of climate change.

Climate change is not acting alone, but instead co-occurring with other human-induced disturbances such as

urbanization. Urbanization is associated with a multitude of environmental impacts, including air pollution, increased foot traffic, clearing habitats for buildings and roads, and the introduction of invasive species. Urbanization results in increased CO<sub>2</sub> emissions from a larger population of people consuming fossil fuels (e.g., from a high density of cars). Additionally, because of the urban heat island, cities have become 1–3 °C warmer than the rural areas surrounding them (Grimmond 2007). Increased temperatures cause more evaporation and surface drying, increasing the length and intensity of drought (Trenberth 2011). In addition to temperature change, deforestation and the clearing of habitats to build cities cause a decrease in C sinks. Soil is a large C sink, and this, combined with increased greenhouse gases, results in decreased C storage (Churkina 2016). Arid and semi-arid ecosystems take up a third of terrestrial land globally and are experiencing rapid urbanization (sensu Hall et al. 2011). As such, the increase in human populations and anthropogenic activities in the Sonoran Desert are contributing to the changes terrestrial ecosystems are experiencing (e.g., Hall et al. 2009; Hamilton and Hartnett 2013; Lohse et al. 2008).

Anthropogenic activities causing urbanization have created an excess of nitrogen (N) that will impact nutrient cycling in ecosystems. Within the last 3 decades, there has been an 8% increase in global inorganic N, and by 2100, this rate is expected to double or triple as a result of increased N deposition, due to fertilizer use and fossil fuel combustion (Lamarque et al. 2005). Consequently, soils in urban environments, including in the Sonoran Desert, have a higher N content than non-urban soils (Cook et al. 2018; Hall et al. 2011). This increase in N may have different impacts depending on the biome in which it is occurring. Though N deposition is an important source of N in soils, an excess can cause changes in nutrient cycling. Nitrogen fertilization can stimulate plant growth and change soil physical and chemical properties, which impacts the soil microbial community (Jia et al. 2020). Because N is often a limiting nutrient in terrestrial ecosystems, increasing N deposition will have negative implications on ecosystems that are adapted to limited nutrients (Baldarelli et al. 2021). There is evidence to support that N fertilization will reduce soil respiration in temperate and boreal forests (Janssens et al. 2010). However, in contrast to this, there is also evidence that supports the stimulation of soil respiration when N is added to soil across all biomes in China (Xiao et al. 2020) and aridland ecosystems (Yang et al. 2022; Zhang et al. 2014). Another study in semi-arid grasslands found that N fertilization had no impact on soil respiration (Osborne et al. 2022). There is, however, still a knowledge gap when looking at how N deposition influences soil respiration in desert ecosystems, especially within the Sonoran Desert. Because of the hot temperatures and limited water that characterizes the Sonoran Desert,

climate factors will likely interact with excess N and other consequences of urbanization, to influence soil respiration.

In arid ecosystems, the interaction between water pulses and other human-induced pulses influence important ecological processes, from material fluxes to species interactions, which can ultimately lead to state transitions (Collins et al. 2014). Most studies investigating the impacts of global climate change on soil focus solely on one factor of change, when, in actuality, factors of change happen simultaneously and interact with one another. It is important to understand how these factors interact to accurately predict the impact of the multiple press and pulse disturbances associated with urbanization that are co-occurring with climate change. The few studies that have looked at multiple changes together are not from deserts and typically focus on the co-occurrence of precipitation and N fertilization, but not temperature, urbanization, or the different precipitation characteristics of pulse size and frequency simultaneously. Often multi-factor studies only manipulate one or some of the factors and rely on natural variability for the others, limiting our ability to understand the relative importance of each factor (Song et al. 2020; Wu et al. 2021). Existing studies that experimentally manipulate both N and precipitation have not focused on the response of soil respiration or soil nutrient availability (Finks et al. 2021; Rillig et al. 2019; Shi et al. 2021).

To study the interactions between N deposition, altered precipitation patterns, urbanization, and increased temperatures to influence the key process of soil respiration and nutrient availability, we subjected urban and exurban soils to differing precipitation regimes (pulse size and frequency), N fertilization, and increased temperature. When considering each human-induced change independently, we predict that urbanization, N fertilization, and increased temperature will stimulate soil respiration and inorganic N availability. Furthermore, we expect soil respiration and N to increase with larger, less-frequent precipitation events. However, when these forms of change are added in combination, we hypothesize that the outcomes will be less predictable. Some positive effects may be intensified when multiple limiting factors are alleviated (such as with the addition of larger precipitation pulses in addition to N), while other combinations may not be additive (if, for example, additional precipitation is not sufficient to overcome water limitation, thereby inhibiting the impact of other concurrent treatments) or even be reversed (such as with the addition of larger precipitation pulses concurrent with inhibition by overfertilization), causing the results to differ from when treatments are applied individually.

## Methods

### Study site

Soil was collected from two sites in the Sonoran Desert. One site was located inside the city of Phoenix, Arizona, at Piestewa Peak (33°33.904'N, 112°0.816'W), and the other from outside the city, at White Tank Regional Park (33°36.290'N, 112° 29.922'W). Both sites are located within the Central Arizona-Phoenix Long-Term Ecological Research (CAP-LTER) project. Soils at these sites are Aridisols, with Typic Haplargids at both sites. CAP-LTER has monitored the N deposition at these sites since 2005. Inside the city, soils receive 8.2 kg N ha<sup>-1</sup> year<sup>-1</sup>, whereas outside the city, soils only receive 6.7 kg N ha<sup>-1</sup> year<sup>-1</sup> (Cook et al. 2018). CAP-LTER has added 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> as solid NH<sub>4</sub>NO<sub>3</sub> to these plots as part of a field fertilization experiment (Hall et al. 2011). Urbanization and N fertilization were studied by collecting soil from four treatments: urban soil with added N, urban soil without added N, exurban soil with added N, and exurban soil without added N.

### Soil collection and experimental design

Soil was collected in bulk from the top 10 cm and placed into a sterile Whirl-Pak bag. Soils were frozen until ready for the experimental incubation. To prepare for a laboratory incubation, soil was sieved to 2 mm to remove rocks, while leaving aggregates intact. 25 ± 0.5 g were placed into 40 mL clear glass vials with a screw-on lid fitted with gas-impermeable septa. Because soil was sieved and poured into a vial, they do not represent field bulk density, and data are expressed per g soil rather than area or volume of soil.

Precipitation was simulated by adding differing amounts of water to the vessels in a fully factorial design. Deionized water was added according to natural precipitation patterns, as well as altered precipitation patterns projected for Phoenix, Arizona. Following Ball et al. (2023), 5 mm pulse events (average summer size for Phoenix over the past 5 years) or 7.5 mm pulse events (50% increase) were simulated either every 2 weeks (simulating the average number of summer events occurring over the past 5 years, spread evenly) or every 4 weeks (reduced frequency). Incubation vessels were placed into an incubator at 30.5°C for 8 weeks to simulate average Phoenix, Arizona temperatures (FCDMC 2022). Vessels were loosely capped to allow for gas exchange. Full-spectrum light was added to the incubator on a timer for daylight (6 a.m. to 6 p.m.). This procedure was repeated on a separate set of

vials incubated at 32.5 °C to simulate projected average Phoenix, Arizona temperatures with a 2 °C increase from future climate change. Therefore, our treatments consisted of a fully factorial design manipulating four global change factors: urbanization (2 levels: urban, exurban), N fertilization (2 levels: control vs. fertilized), precipitation (4 levels: 5 mm and 7.5 mm pulse sizes at both every 2 and 4 weeks), and temperature (2 levels: 30.5 and 32.5 °C). Each treatment combination was replicated four times, yielding 128 separate incubation vials.

### Soil measurements

CO<sub>2</sub> flux (soil respiration) was measured in incubation vessels using an LI-COR Infrared Gas Analyzer (LI-7000, LI-COR, Lincoln, NE) regularly for the duration of the 8-week period. Following Ball et al. (2015), vessels were capped tightly and flushed with CO<sub>2</sub>-free air for 45 s at 5 psi. Sealed vessels were placed back in the incubator for 5–8 h. 2 mL of headspace gas, including respired CO<sub>2</sub>, from each vessel was removed with a syringe and injected into the LI-COR Gas Analyzer. To calculate CO<sub>2</sub> concentration, the maximum integration value (area under the curve) was recorded after every sample injection and compared against a standard curve. Net C mineralization was then calculated from the CO<sub>2</sub> concentration using the equation

$$(AW_C * [CO_2] * V) / (R * T) / (S * d),$$

where  $AW_C$  is the atomic weight of C (12 g mol<sup>-1</sup>),  $V$  is the headspace volume,  $R$  is the gas constant (0.082 L atm K<sup>-1</sup> mol<sup>-1</sup>),  $T$  is the temperature,  $S$  is the initial dry soil mass added to the vials, and  $d$  is the days of CO<sub>2</sub> accumulation. After measurement, vessels were then opened to the atmosphere and placed back into the incubator with their caps loosened. This process was repeated throughout the incubation, initially every other day to capture the initial flush of C mineralization, becoming less frequent during the later stages of the incubation. Cumulative CO<sub>2</sub> release was calculated for the entire 8-week incubation, assuming a linear change in the rate between sampling days.

After the 8-week incubation period, soil water content (SWC) was measured. 5 ± 0.5 g of soil was taken from each incubation vessel and dried at 105 °C for 48 h. To measure inorganic N (NO<sub>3</sub> + NO<sub>2</sub>-N, abbreviated from here as NO<sub>y</sub>, and NH<sub>4</sub>-N), 10 ± 0.5 g of fresh soil was extracted into 50 mL 2 M KCl. Samples were centrifuged at 15,000 rpm for 10 min, then frozen until run on a flow injection analyzer (Lachat QC8000), and expressed as the amount of N per g of dry-equivalent soil (Ball and Alvarez Guevara 2015).

### Data analyses

Data are publicly available (Ball 2023), and all statistical analyses were conducted in R (version 4.0.2, The R Foundation). C mineralization data were analyzed using analysis of covariance (ANCOVA) testing for the effects of site (urbanization: urban vs exurban), fertilization (+N or control), Precipitation (5 mm × 2wks, 5 mm × 4wks, 7.5 mm × 2wks, or 7.5 mm × 4wks), and temperature (30.5 or 32.5 °C). Days of incubation was included as a covariate to account for repeated testing on the same experimental incubation vessels over time (Wan 2019). The interaction of these factors was also included in the model. Soil inorganic N data were only measured at the end of the experiment, and thus were analyzed with a 4-way analysis of variance (ANOVA) of Site, Fertilization, Precipitation, and Temperature, as well as their interactions. For both C mineralization and inorganic N data, where the main effect of precipitation was significant, a post hoc Tukey HSD test was conducted using the package *agricolae* to determine significant differences among the four precipitation treatments.

### Results

All of the main effects, except temperature, significantly influenced respiration rates, with many significant interactions among them (Table 1). The three-way interaction of Site:Temperature:Day was significant, suggesting that the way urban versus exurban sites differed in respiration depended on the temperature, with the influence of temperature changing over time (Fig. 1). Soil from the urban site had higher initial respiration rates than the exurban soils, particularly at 30.5 °C where urban soil respiration rates were ~3 × higher on day 1 (52.3 ± 6.0 vs. 17.8 ± 2.2 μmol CO<sub>2</sub> g<sup>-1</sup> soil d<sup>-1</sup>). The difference diminished over time, but this pattern was also true when considering cumulative respiration across the incubation (Site:Temperature in Table 2; Fig. 2). All soils incubated at 30.5 °C saw a small increase in respiration around day 35 of incubation; however, this was not apparent in soils incubated at 32.5 °C. Additionally, urban soils with added N initially had the highest respiration rates compared to the other treatments (52.3 ± 5.8 μmol CO<sub>2</sub> g<sup>-1</sup> soil d<sup>-1</sup> compared to 23.0 ± 1.4 across other treatments). Further, precipitation had significant 2-way interactions with both site and fertilization (Table 1), wherein the post hoc pairwise test showed that soils receiving the 7.5 mm × 4wk pulses respired more than the other three precipitation treatments, particularly in the urban soils and with N fertilization. However, the precipitation treatment that increased cumulative respiration depended on urbanization, temperature, and fertilization (significant Site:Temperature:Precipitation and Fertilization:Precipitation interactions).



**Table 1** *P* values resulting from the Analysis of Covariance (ANCOVA) testing the impacts of site, fertilization, precipitation treatment, and temperature, with day as a continuous variable, as well as their interactions, on soil respiration

	<i>P</i> value
Site	0.021
Fertilization	< 0.001
Precipitation	< 0.001
Temperature	0.737
Day	< 0.001
Site:fertilization	0.042
Site:precipitation	0.004
Fertilization:precipitation	< 0.001
Site:temperature	< 0.001
Fertilization:temperature	0.221
Precipitation:temperature	0.400
Site:day	0.331
Fertilization:day	< 0.001
Precipitation:day	< 0.001
Temperature:day	0.298
Site:fertilization:precipitation	0.788
Site:fertilization:temperature	0.118
site:precipitation:temperature	0.054
Fertilization:precipitation:temperature	0.241
Site:fertilization:day	0.066
Site:precipitation:day	0.063
Fertilization:precipitation:day	0.416
Site:temperature:day	< 0.001
Fertilization:temperature:day	0.745
Precipitation:temperature:day	0.246
Site:fertilization:precipitation:temperature	0.891
Site:fertilization:precipitation:day	0.444
Site:fertilization:temperature:day	0.477
Site:precipitation:temperature:day	0.757
Fertilization:precipitation:temperature:day	0.797
Site:fertilization:precipitation:temperature:day	0.793

Cumulative respiration was generally higher under the 7.5 × 4 pulse and lower under the 5 × 4 pulse ( $208.0 \pm 13.9$  vs  $169.5 \pm 16.6$   $\mu\text{mol CO}_2 \text{ g}^{-1}$  soil, respectively), but this was not the case for all urbanization × temperature × fertilization combinations.

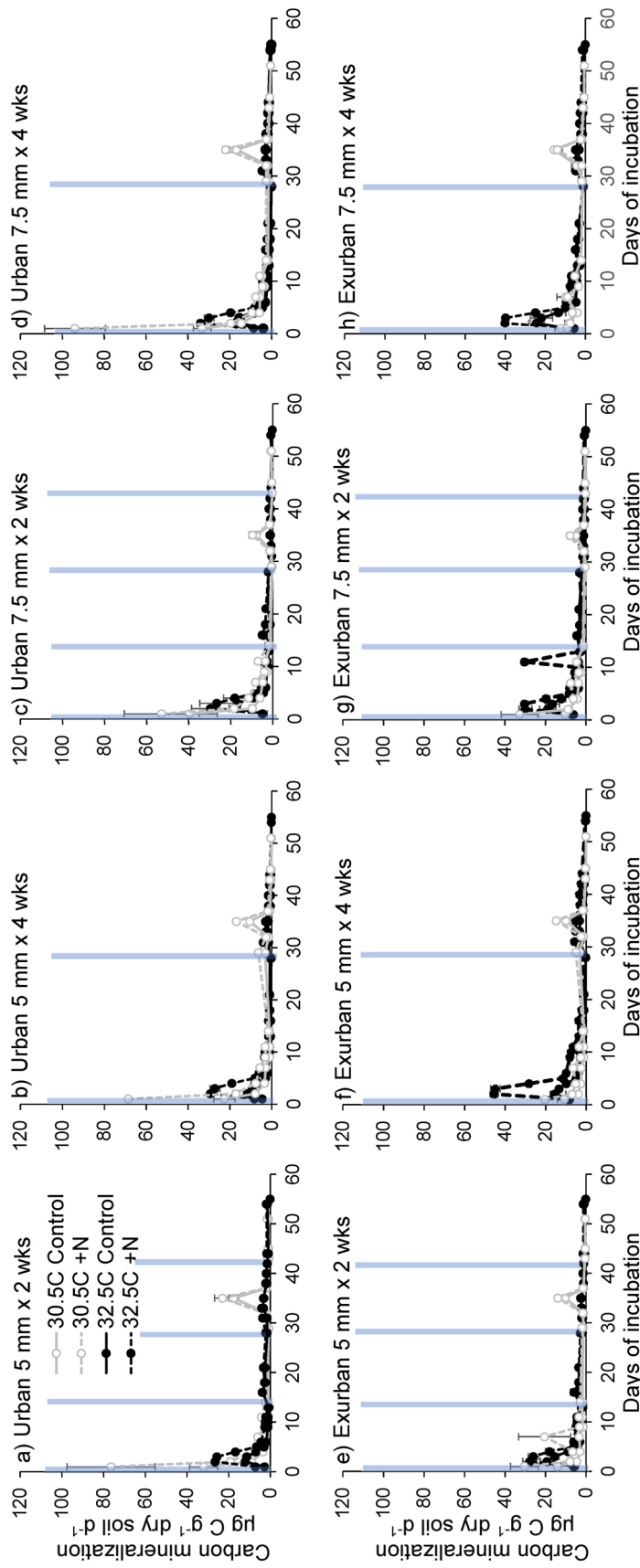
Nitrate and nitrite ( $\text{NO}_y$ ) levels were significantly impacted by all of the main effects (Table 2). The four-way interaction between all of the factors was also significant. Therefore, the way that soil N responded to any one of the global change factors (precipitation, temperature, urbanization, and N fertilization) depended on the level of the other factors. Overall, the urban soils contained 2 × more  $\text{NO}_y$  than did the exurban soils (Fig. 3). Samples taken from plots with added N had > 2 × higher  $\text{NO}_y$  levels than the control plots, regardless of which site they were taken from. The

post hoc test showed that  $\text{NO}_y$  levels were highest under the 7.5 mm × 4wk treatment, and lowest in the 7.5 mm × 2wk treatment when the soil became water-logged. Exurban soils incubated at 32.5 °C and receiving 5 mm × 4wk pulse events had higher  $\text{NO}_y$  levels than those incubated at 30.5 °C. Urban soils receiving 5 mm × 2wk or 7.5 mm × 4wk pulse events had the highest  $\text{NO}_y$  levels.

All of the main effects except site were significant factors in ammonium levels in the samples (Table 2). At both sites, the post hoc test showed that soils that received the 7.5 mm × 2wk and became water-logged had 10 × higher ammonium levels than the other precipitation treatments. The ammonium in samples under this treatment surpassed  $\text{NO}_y$  levels, which are the only samples that experienced this result. All other treatments had relatively low ammonium levels compared to  $\text{NO}_y$ . The post hoc tests also revealed that ammonium levels significantly decreased in the order of 7.5 mm × 2wks > 5 mm × 2wks > 7.5 mm × 4wks > 5 mm × 4 wks, corresponding to a decrease in ammonium with lower total amount of added water. Additionally, there were three significant three-way interactions that describe the complex responses of ammonium across the treatments: Site:Fertilization:Precipitation, Site:Precipitation:Temperature, and Fertilization:Precipitation:Temperature (Table 2, Fig. 3). The first interaction shows that fertilization significantly increased ammonium only under the 5 × 2 and 7.5 × 2 precipitation treatments in the exurban site, while it decreased ammonium under the 5 × 2 and 7.5 × 2 treatments at the urban site. The second explains that the way site influenced ammonium levels depended on the precipitation treatment and temperature. Generally, the soils incubated at 32.5 °C had slightly higher ammonium levels than those incubated at 30.5 °C, particularly at the exurban site. The third significant three-way interaction explains that the way fertilization influences ammonium levels varies across the precipitation treatments and temperature, where in some cases, fertilization increases ammonium levels compared to the control, but in other cases, fertilization decreases ammonium.

## Discussion

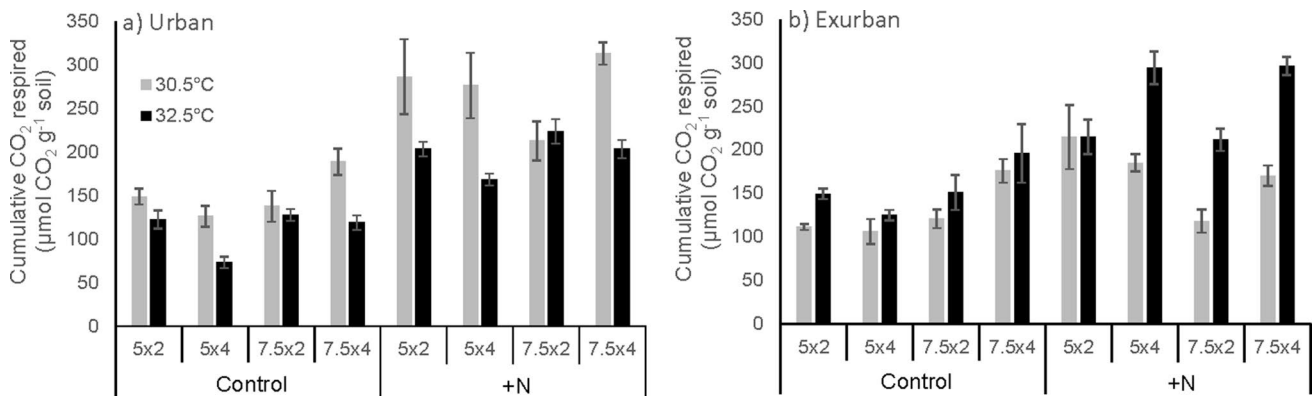
We hypothesized that both inorganic N concentrations and soil respiration rates would increase under elevated N, urbanization, increased temperatures, and less-frequent but larger precipitation events. As detailed below, not all of these predictions were supported. All of these factors were manipulated simultaneously, so we could better understand how they interact with each other to change soil processes. The prevalence of significant interactions among the main effects for both soil respiration and mineral N supports our hypothesis that the biogeochemical responses to multiple co-occurring forms of global change will differ than when



**Fig. 1** Soil respiration levels from soils from an urban site (Piestewa Peak; **a–d**) versus an exurban site (White Tank Mountains; **e–h**) that were incubated in a factorial experiment of precipitation amount (5 or 7.5 mm), precipitation frequency (every 2 or 4 weeks), N fertilization (+N vs control), and temperatures (30.5 or 32.5 °C). Values are mean ± SE. Blue bars designate days when a precipitation treatment was added

**Table 2** *P* values resulting from the Analysis of Variance (ANOVA) testing the impacts of site, fertilization, temperature, and precipitation, as well as their interactions, on mineral nitrogen in soil at the end of the laboratory incubation. Mean values  $\pm$  SE for each treatment are provided in Online Resource 1

	NO <sub>2</sub> + NO <sub>3</sub> -N <i>P</i> value	NH <sub>4</sub> -N <i>P</i> value	Cumulative respiration
Site	< 0.001	0.490	0.521
Fertilization	< 0.001	0.001	< 0.001
Temperature	< 0.001	< 0.001	0.971
Precipitation	< 0.001	< 0.001	< 0.001
Site:fertilization	< 0.001	< 0.001	0.002
Site:temperature	0.019	< 0.001	< 0.001
Fertilization:temperature	0.301	0.020	0.048
site:precipitation	< 0.001	< 0.001	0.020
Fertilization:precipitation	0.006	0.608	< 0.001
Precipitation:temperature	< 0.001	< 0.001	0.009
Site:fertilization:temperature	0.065	0.759	0.149
Site:fertilization:precipitation	< 0.001	< 0.001	0.338
Site: temperature: precipitation	0.203	< 0.001	0.037
Fertilization:temperature:precipitation	0.056	< 0.001	0.060
Site: fertilization: precipitation: temperature	< 0.001	0.747	0.416



**Fig. 2** Cumulative CO<sub>2</sub> respired over the duration of the 8 week incubation of soils from an urban site (Piestewa Peak; **a**) versus an exurban site (White Tank Mountains; **b**) that were incubated in a facto-

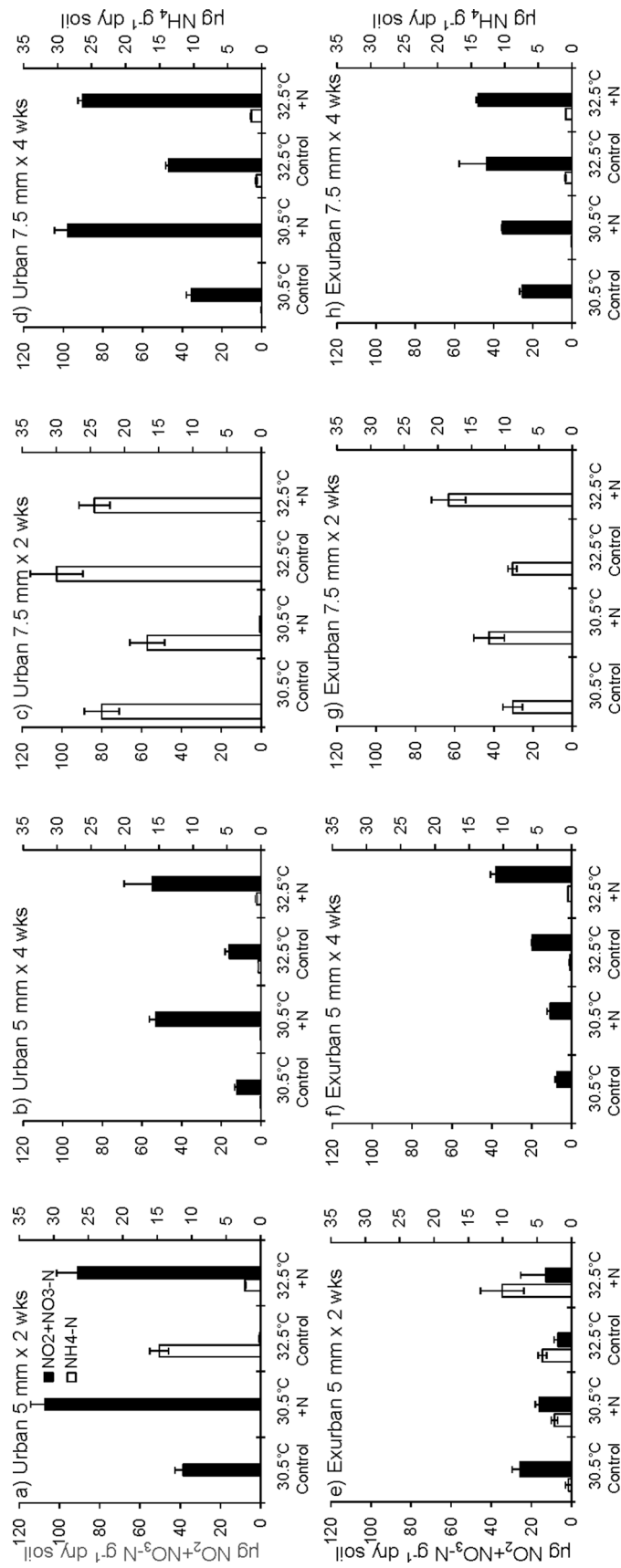
rial experiment of precipitation amount (5 or 7.5 mm), precipitation frequency (every 2 or 4 weeks), N fertilization (+N vs control), and temperatures (30.5 or 32.5 °C). Values are mean  $\pm$  SE

considered individually. We investigate these main effects and interactions below by discussing each of the environmental factors in turn.

### Urbanization

Urban soils had higher N levels than exurban soils. This is likely due to increased N deposition in the city compared to the exurban environment (Cook et al. 2018). A similar study done on soils in the Sonoran Desert also found that urban soils had a higher concentration of inorganic N than did their exurban counterpart (Hall et al. 2009). Additionally, urban soils experienced increased respiration rates, particularly at current average temperatures (30.5 °C; Figs. 1 & 2), which supports our hypothesis that the additional N would stimulate soil activity.

N deposition will likely have impacts on soil interactions, especially at the N levels that our study finds within the city of Phoenix (Cook et al. 2018). The continual population growth expected in Phoenix will put more pressure on resources, such as water, that ecological communities rely on. Additionally, the “urban” versus “exurban” designation incorporates many different stressors beyond N, such as other forms of air pollution, introduced species, and physical disturbances that can structure distinct soil communities that differ in their physiological capabilities (Gallas and Pavao-Zuckerman 2022).



**Fig. 3** Inorganic nitrogen levels in soils from an urban site (Piestewa Peak; **a–d**) versus an exurban site (White Tank Mountains; **e–h**) that were incubated in a factorial experiment of precipitation amount (5 or 7.5 mm), precipitation frequency (every 2 or 4 weeks), N fertilization (+N vs control), and temperature (30.5 or 32.5 °C). Values are mean  $\pm$  SE. Note that the values for  $\text{NO}_2+\text{NO}_3\text{-N}$  are on the left vertical axis, while values for  $\text{NH}_4\text{-N}$  are on the right



## Fertilization

The addition of N to urban and exurban soils increased inorganic N in both sites, compared to control plots with no added N. Soils taken from plots with added N also had higher respiration rates. This agrees with the other studies that found that the addition of N stimulated soil respiration (Comeau et al. 2016; Fang et al. 2017), but disagrees with studies finding either no response (Rowlings et al. 2013) or a decrease in soil respiration rates (Ding et al. 2007). Fang et al. (2017) concluded that the stimulation in soil respiration may be a short-term response, and could decline over the long term. However, the N added to soils in our study has been applied annually since the early 2000s, indicating that microbes are not adjusting to the added N and returning to their unamended levels of activity. Experimental fertilization rates in these plots are significantly higher than ambient deposition (Cook et al. 2018), and it is possible that this higher rate induces a larger and longer sustained response than would happen under lower levels of atmospheric deposition. However, the stimulated respiration we measured from our high fertilization rate suggests that co-limitation by C or P would not limit the impact of lower levels of ambient deposition. While there has yet to be a complete consensus on the effects added N has on soil respiration, especially in environments that have limited data available such as the Sonoran Desert, the results of our study suggest an increase. While soil pH was not measured in this study, past data from these sites show that pH under long-term N addition is only slightly lower than control soils, but both are in the range of neutral and differ by only by a magnitude of 0.2–0.5 with limited ecological impacts (Hall et al. 2011), suggesting that acidification is not playing a major role in the response we measured.

## Precipitation

Receiving moderate amounts of water (7.5 mm × 4wk and 5 mm × 2wk pulse events) increased  $\text{NO}_y$  over the treatment receiving the least amount of water. However, soils receiving the 7.5 mm × 2wk precipitation became waterlogged and likely anaerobic. Though we did not measure oxygen status in this experiment, anoxic conditions could result in  $\text{NO}_y$  being consumed through anaerobic processes such as denitrification or dissimilatory nitrate reduction to ammonium (DNRA). The loss of  $\text{NO}_y$  and buildup of ammonium that we observed in the 7.5 × 2 treatment could be the result of DNRA. A previous meta-analysis demonstrates that DNRA is positively correlated with precipitation and temperature, which supports our measurements

of higher soil  $\text{NH}_4^+$  in the wettest soils under the warmer temperatures (Cheng et al. 2022). Interestingly, however, exurban soils receiving N fertilization contained more  $\text{NH}_4^+$  at the end of the incubation, reflecting the initially greater amount of  $\text{NO}_y$  utilized to generate the  $\text{NH}_4^+$ , but urban soils receiving N fertilization contained less  $\text{NH}_4^+$  than the control soils. Our data are not able to parse the mechanism for this, but one possibility is that denitrifying microbes were more abundant in these high-N-fertilized, urban soils and were more effectively able to compete for  $\text{NO}_y$  than in the other treatments.

In the natural environment, however, soil is able to drain, so it is unlikely that the soil would become anaerobic for any significant period of time. Similarly, soils with the highest respiration rates were those that received 7.5 mm × 4wk pulses. This is what is predicted with climate change, meaning we can expect more soil respiration as summer precipitation events increase in size and are reduced in frequency. Because the incubation vessels were loosely covered and incubated at average annual temperatures (rather than summer temperatures), evaporation between pulse events would not be to the same extent as a natural setting during the Sonoran Desert monsoon season. Therefore, our simulated precipitation was able to maintain soil moisture longer than would happen in the field. Antecedent soil moisture influences the impact of rainfall pulses (e.g., Cable et al. 2008; Harms and Grimm 2012), which suggests a potentially dampened influence of our rainfall pulse simulations compared to a natural setting. Yet, our results reflect the impact of water limitation in these soils, and therefore a conservative impact of altered precipitation that changes the duration of soil moisture between pulses. Some studies suggest that altered precipitation impacts respiration rates could be due to changes in the soil microbial community because microbial activity is positively correlated with precipitation amount (Wang et al. 2021). Additionally, altered precipitation pulses can alter the microbial community and therefore their associated enzyme activity (Wang et al. 2021; Zhang et al. 2021). For example, Zhang et al. (2021) found decreased rather than increased  $\text{CO}_2$  flux with reduced frequency of larger precipitation pulses, associated with reduced *Vicinamibacteria*, *Verrucomicrobiae*, and *Blastocatellia* abundance and concomitant decreases in glucosidase activity.

## Temperature

Temperature influenced ammonium levels in both urban and exurban soils. Soils incubated at 32.5 °C had higher ammonium levels than soils incubated at 30.5 °C. Temperature also influenced  $\text{NO}_y$  levels, though the influence is not as straightforward as with ammonium. Exurban soils had more inorganic N when incubated at 32.5 °C and receiving the 5 mm × 4wk

precipitation, compared to those incubated at 30.5 °C. One study agreed that inorganic N levels increase with an increase in temperature (Luo et al. 2013). However, a few studies have explored the connection between temperature and N levels in arid and semi-arid environments, resulting in a large gap in knowledge on this topic. In our study, a 2 °C increase in temperature did not significantly influence soil respiration rates. This finding is similar to some studies and in contrast to others. Liu et al. (2009) reported a reduction of soil respiration in semi-arid regions experiencing warming temperatures. Other studies done in desert and steppe ecosystems in China concluded that increased temperatures had little effect on soil respiration (Liu et al. 2016; Wang et al. 2019a), which are most similar to the results of this study. It is possible that Sonoran Desert soil microbes, which are adapted to tolerate high temperatures, are not sensitive to a relatively small 2 °C increase like microbes in more temperate or cold ecosystems that would likely have lower heat tolerance (e.g., Schindlbacher et al. 2009; Wang et al. 2019a, 2014b; Zou et al. 2018). Notably, though there is no direct influence of temperature alone, temperature does significantly interact with other factors to influence respiration. For example, warmer temperatures slightly reduce cumulative respiration in the urban soils, but at the exurban site cumulative respiration is stimulated by the 2° increase, particularly under N fertilization (Fig. 2).

The late-stage peak of C mineralization in the 30.5 °C incubation has been seen in other microcosm studies, particularly with sandy soils of low clay content (Ball et al. 2018; Haddix et al. 2011; Tian et al. 2014). The cause is uncertain, but may result from a short pulse of labile C from microbial death, release of protected C from soil aggregates, or a temporary shift to another more recalcitrant form of C after labile C was consumed. This peak tended to be greater in fertilized soil than unfertilized, and urban soils had a larger peak than exurban soils when ample water was added. Such N-limitation suggests the use of a carbon-rich food source rather than a nitrogen-rich source, and that the source is not likely a physical process such as degassing of soil carbonates. Because the peak only lasted one sampling day, it is possible that the pulse occurred in the 32.5 °C incubation but was not captured by the timing of our measurements. Alternatively, a small but sustained peak occurred in the 32.5 °C incubation over 31–35 d, which might correspond to the water addition at day 28 or a similar shift to a new C source with a slower but more sustained mineralization rate.

### Fertilization and precipitation on NO<sub>y</sub> and soil respiration

The stimulation of respiration with moderate precipitation amounts was enhanced when N was added. This shows that the interaction between fertilization and precipitation

enhances the way the soil responds, compared to each factor alone. Cumulative soil respiration was stimulated by both fertilizer (+ 65% relative to the control) and precipitation (+ 15% relative to the control) treatments, but when these two global change factors were added together, we observed the highest cumulative soil respiration (+ 85% relative to the control). This is consistent with similar studies done on the interaction between fertilization and precipitation on soil respiration. Since respiration from N-fertilized soils increases with increasing precipitation, then it is likely that water is the limiting factor (Wang et al. 2019b).

Similar to this study, Sanchez-Martin et al. (2010) found that the addition of N fertilization in conjunction with increased precipitation lead to a significant increase in inorganic N. Climate change and urbanization are expected to cause higher amounts of N in soils, as well as larger rain pulses less frequently. As a result of this, we can expect inorganic N levels in soils to increase as rainfall changes and N deposition increases.

### Site, fertilization, and precipitation on NO<sub>y</sub>

Soils taken from urban plots with added N had higher NO<sub>y</sub> levels than other soils, regardless of precipitation. In contrast, the exurban soils were more impacted by precipitation than by the addition of N. Of the exurban soils, the samples receiving the 7.5 mm × 4wk pulses produced the most NO<sub>y</sub>, likely because they were getting ample water to stimulate activity but not excessive water to create anaerobic conditions.

### Conclusions

These findings lead us to conclude that urbanization will cause an increase in NO<sub>y</sub> levels and potentially C loss from respiration. The larger but less-frequent pulses that are expected with climate change (simulated in the 7.5 mm × 4wk treatment) will cause soil microorganisms to respire more and produce more NO<sub>y</sub>. Because soil respiration is a major flux of CO<sub>2</sub> into the atmosphere, increasing this flux will result in less C stored in the soil and more CO<sub>2</sub> in the atmosphere. All of the factors discussed in this study have the potential to increase soil respiration and, therefore, enhance the anthropogenic disturbances to the C cycle.

An increase in inorganic N levels in soils is likely to cause N pollution in water. Runoff during large rainfall events could result in more N leaking into the nearby aquatic ecosystems. As this study has shown, we will see the highest NO<sub>y</sub> levels in urban areas, where large amounts of people live close together in one area. Cities may then face significant impacts from excess N contaminating groundwater and

causing negative impacts on humans and the environment as a whole.

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**Author contribution statement** MW and BB conceived and designed the experiment. MW performed the experiments. MW and BB analyzed the data. MW wrote the manuscript with editorial advice from BB.

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**Data availability** The datasets generated during the current study are publicly available on the EDI Data Portal.

**Code availability** Not applicable.

## Declarations

**Conflicts of interest** The authors have no conflicts or competing interests.

**Ethical approval** Ethics approval was not required for this study.

**Consent to participate** No human subjects were involved in this research.

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