



## Altered nitrogen and precipitation along urban gradients affect harvester ants and seed sources



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### ABSTRACT

We investigated the effects of nitrogen deposition and precipitation on *Messor pergandei* (Mayr) harvester ants and plants to identify alterations in the desert food web in California. We measured ant colony attributes and shrub fruit densities, as well as nitrogen and carbon concentrations and stable isotopes, at 18 sites along a nitrogen deposition gradient. Ant nest density increased from low to high deposition sites; however, ant nest mound size and the density of abandoned nests decreased as deposition increased. Nest mound size was positively correlated with the size and age of the colony; therefore, these results suggest that colonization has been more frequent with increased inter-colony competition in areas of high deposition. Nitrogen and carbon isotope values of perennial plant leaves and seeds, annual plant seeds, and ants were significantly enriched in the heavy isotopes from low to high nitrogen deposition regions, indicating the possibility of plants assimilating different sources of both elements, including anthropogenically-produced compounds. Plant carbon isotope discrimination also differed with the decrease in precipitation across the gradient. Considering that deserts are limited by both nitrogen and precipitation, our results suggest that altered nitrogen inputs in conjunction with precipitation may result in cascading effects through trophic levels and drive arid ecosystem change.

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### 1. Introduction

Global changes, including atmospheric nitrogen deposition and altered precipitation regimes, are driving novel ecosystem responses. Nitrogen deposition can affect soil microbial processes and plant available nitrogen producing alterations in ecosystem structure and function (Fenn et al., 2003a; Rao et al., 2009; Sirulnik et al., 2007; Vourlitis et al., 2007). A synthesis by Bobbink et al. (2010) found that nitrogen is more critical in determining community composition than previously realized. These alterations typically result in increases in nitrogen availability that can cause weedy plant invasions in desert systems, altering fire regimes and plant community composition (D'Antonio and Vitousek, 1992; Evans et al., 2001; Schwinning et al., 2005). Most current studies focus on the effects of nitrogen deposition on one trophic level or

taxonomic group; few studies examine the cascading effects of nitrogen deposition across trophic levels.

The arid western United States experiences primarily dry nitrogen deposition, which has dramatically increased over the last several decades from sources such as motor vehicle exhaust, power plant emissions, industrial emissions, agricultural field fertilization, and feedlots (Fenn et al., 2003a). For example, Los Angeles, California, USA, and nearby population centres create substantial amounts of pollution, including nitrogenous compounds from motor vehicle exhaust, each year through fossil fuel combustion (Fenn et al., 2003b; Allen et al., 2009). The nitrogen is carried across southern California and falls out as winds move east, creating a nitrogen deposition gradient. This nitrogen deposition gradient extends about 150 km across the Coachella Valley and Joshua Tree National Park, providing a unique opportunity to examine the ecological effects of such a gradient. Rao et al. (2009) and Allen et al. (2009) detailed changes in soil processes and annual plant communities following large pulses of nitrogen fertilizer within this gradient; however, it is not known whether these changes have influenced higher trophic levels.

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The effects of an altered hydrologic and nitrogen cycle in the Mojave and Colorado Desert systems are complex and not well known. If declines in rainfall and soil moisture reduce nitrogen availability, plant populations and the animals that depend on them could be negatively impacted. Conversely, increases in nitrogen deposition could mitigate reductions in microbially-produced nitrogen in low precipitation environments (Vourlitis et al., 2007).

Little is known about seed production and subsequent granivory under elevated nitrogen (Throop and Lerdau, 2004). Higher nitrogen levels typically result in increased plant biomass production and higher tissue nitrogen concentrations, which can regulate trophic structure (Cebrian et al., 2009). Throop and Lerdau (2004) found higher growth or consumption rates in leaf-feeding insects with increased nitrogen in the majority of twenty studies reviewed. Considering that nitrogen is highly limiting for most consumers, plants producing more seed or seed with higher nitrogen concentrations could cause an increase in populations of granivores, including harvester ants (Throop and Lerdau, 2004). Granivory and seed movement can deplete plant populations and/or shift species composition (MacMahon et al., 2000; White and Robertson, 2010). Thus, how plants and granivores react to nitrogen deposition is critical as the effects of increased nitrogen on plant and seed characteristics could have large consequences for desert ecosystems (Kelrick et al., 1986).

Harvester ants, birds, and rodents move, store, and consume mass quantities of seed (MacMahon et al., 2000; White and Robertson, 2010). The harvester ant, *Messor pergandei* (Mayr), is a ubiquitous and important granivore in the deserts of southern California and is co-dominant with *Pogonomyrmex* species (Went et al., 1972). Seeds from annual forbs and grasses are typically harvested, but perennial shrub and grass seeds are also collected (Went et al., 1972; Wissinger, personal observation). Seed refuse, flowers, stems, and other debris are brought from inside the nest and deposited around the nest entrance, creating large mounds (Went et al., 1972). The size of these mounds is correlated with the size and age of the ant colony inhabiting the nest (Gordon, 1984; Wagner and Gordon, 1997).

Considering the significance of harvester ants in desert ecosystems, it is essential to understand the effects of altered nitrogen and precipitation on this key species and its food sources. We investigated how atmospheric nitrogen deposition and precipitation affected multiple trophic levels, including plants, seeds, and seed-harvesting ants across a nitrogen deposition and precipitation gradient in the Coachella Valley and Joshua Tree National Park, California. The use of stable isotopes enabled us to track nitrogen and carbon assimilation by the organism in successive trophic levels. Our study asked: 1) Are harvester ant nest densities greater in areas with higher nitrogen deposition and precipitation? 2) Does ant nest size change in areas with higher nitrogen deposition and precipitation? 3) Is seed production increased with higher nitrogen deposition and precipitation? 4) Do areas of higher nitrogen deposition and precipitation alter  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and percent carbon and nitrogen across trophic levels?

## 2. Materials and methods

### 2.1. Site selection and description

We examined 18 sites across a nitrogen (N) deposition gradient located in the Colorado and Mojave deserts, which stretched from the west end of the Coachella Valley to the east side of Joshua Tree National Park (Figure A1, Table A1). Nine sites were within the park boundaries, while another nine sites were situated among urban areas between Interstate 10 and the park. We selected sites based

on the presence of *M. pergandei* ants and their inclusion within an established atmospheric nitrogen deposition gradient (Allen et al., 2009; Rao et al., 2009). For this study, nitrogen deposition values were obtained from a Models-3/Community Multiscale Air Quality (CMAQ) model (Fenn et al., 2003b; Tonnesen et al., 2007) and  $\text{HNO}_3$  atmospheric concentrations from 2010 to 2011 sampling efforts (Bell, 2012).

Nitrogen deposition gradients are often accompanied by other abiotic gradients (Hall et al., 2011; Rao and Allen, 2010). In order to account for other possible gradients, we also determined annual precipitation, atmospheric  $\text{CO}_2$  concentrations, and soil nitrogen across our study sites. Annual precipitation values from 2001 to 2011 for each site were obtained from the PRISM model and were averaged (PRISM Climate Group, 2004, Fig. 2). Atmospheric  $\text{CO}_2$  concentrations were of interest because *Larrea tridentata* may increase nitrogen uptake under high  $\text{CO}_2$  conditions (Jin and Evans, 2010). Additionally, elevated atmospheric  $\text{CO}_2$  concentrations affect water use efficiency in many plants (Farquhar et al., 1989). Thus, atmospheric  $\text{CO}_2$  concentrations and  $^{13}\text{C}$  values for background atmospheric  $\text{CO}_2$  were measured by taking three air samples from each of six representative sites (two sites each from the west end, middle, and east end) across the gradient. Air was collected in vials during the early afternoon and stored until analysis on a Gasbench II (Thermo Finnigan) coupled with a continuous-flow isotope-ratioing mass spectrometer (Thermo Finnigan DELTA plus) at the University of Idaho Stable Isotopes Laboratory. To determine soil nitrogen at each site, we collected ten soil samples five centimeters deep and thoroughly mixed them in the field. At the University of Idaho, soil nitrogen in the forms of nitrate.

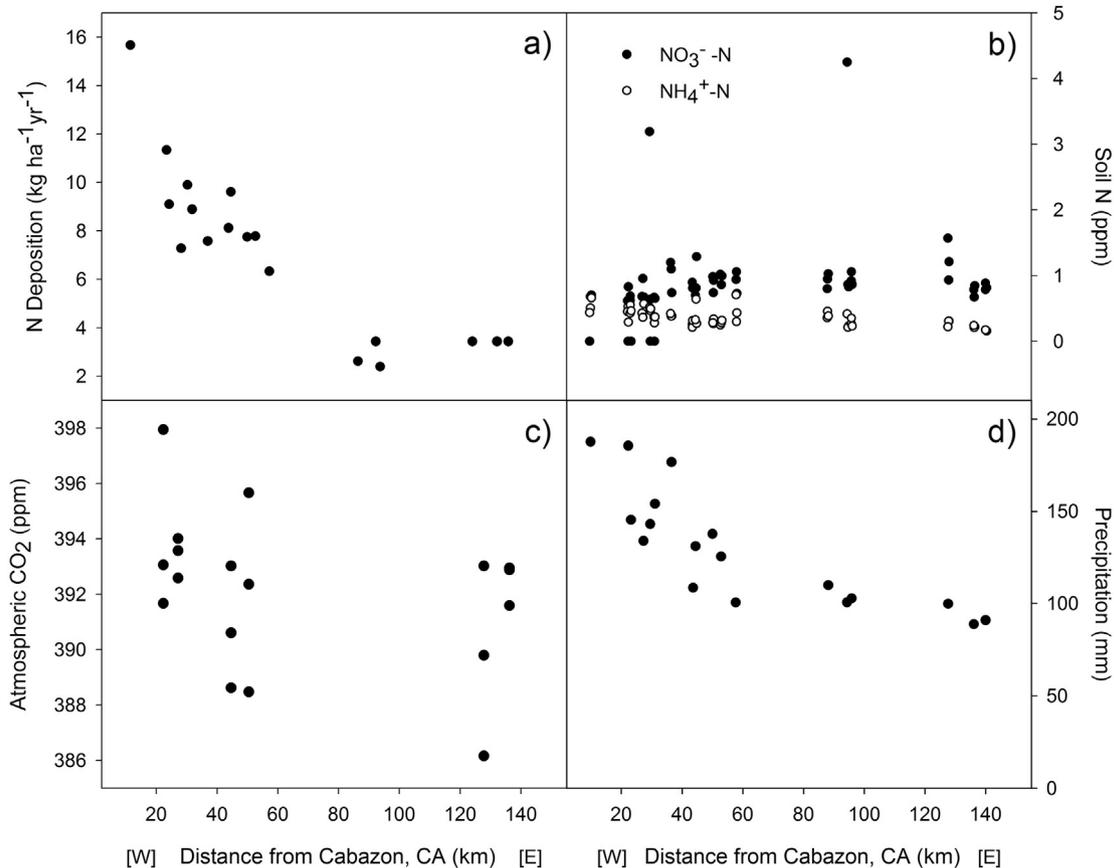
( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) was extracted using potassium chloride (KCl). The resulting solutions were evaluated by flow injection analysis at the Soil and Plant Analysis Laboratory, Brigham Young University, Provo, Utah.

The sites were located in *L. tridentata* (DC.) Coville (evergreen shrub)/*Ambrosia dumosa* (A. Gray) (drought-deciduous shrub) vegetation communities. Other co-occurring native shrubs included *Encelia farinosa* (A. Gray ex Torr.) and *Hymenoclea salsola* (A. Gray). Common native annual forbs such as *Cryptantha*, *Coryphantha*, *Eriodinium*, and many *Aster* species were present with non-native grasses (*Schismus* spp. and *Bromus rubens* L.) and non-native forbs (e.g., *Brassica tournefortii* Gouan). Soil texture at each site was either loamy sand or sand. Sites were located in sandy washes, basins, or bajadas, and ranged from approximately 90–715 m above mean sea level (Table A1). Sites are described as the distance from Cabazon, California, USA, where sites closest to Cabazon (west side of study area) have the highest nitrogen deposition and annual precipitation; sites furthest from Cabazon (east side of study area) have the lowest nitrogen deposition and annual precipitation.

### 2.2. Field measurements

At each site across the gradient, plant and harvester ant measurements were taken on two perpendicular transects intersecting at their midpoints to form crosses. We established five crosses per site and the center point of each cross was separated by 300 m. Each segment of the cross was 100 m long by 4 m wide, which allowed us to survey 1600  $\text{m}^2$ . Thus, at each site we surveyed a total of 8000  $\text{m}^2$ .

For three of the five crosses at each site, we measured shrub density and counted fruits and seeds. All *A. dumosa* and *L. tridentata* shrubs were counted within the 1600  $\text{m}^2$  sample area. Fruit and seed counts were obtained from 20 individual shrubs of each species in each cross from shrubs nearest the centerline at 20, 40,



**Fig. 1.** Nitrogen deposition (a), soil nitrogen (b), values for each of 18 sites and atmospheric CO<sub>2</sub> concentrations (c) and precipitation (ten year average)(d) for six of the sites across the Coachella Valley and Joshua Tree National Park, CA. Each site is measured as the distance from Cabazon, CA, which is the farthest town on the west side of the gradient.

60, 80, and 100 m. Fruit and seed counts were sampled from the terminal 15 cm of the branch closest to the centerline. Approximately 100 g each of leaves and seeds from randomly selected *A. dumosa* and *L. tridentata* within each cross were collected, composited, air-dried, and stored in the laboratory. Seeds were also collected from the following annual plants, which were harvested by the ants (Wissinger, *personal observation*): *Aster* spp. *B. tournefortii*, *Cryptantha* spp., *Corzanthe* spp., and *Eriodinium* spp. Since annual plant species presence varied across sites, two species were chosen based on visual observation of ants collecting seeds at each site.

*Messor pergandei* nest density was calculated by counting nests with active ants within each cross. Nests no longer in use surrounding the active colony were considered abandoned nests. We measured active nest mound diameter at its widest point and nest mound height at its highest point. We collected ants from at least three randomly chosen nests at each cross, created a composite sample, and preserved ant samples in 70% ethanol.

### 2.3. Lab analyses

In the laboratory, all plant and ant samples were cleaned, rinsed with deionized water, oven dried (50 °C), and stored in airtight vials until analysis. Ant gasters were removed to eliminate undigested plant material from influencing the carbon and nitrogen analysis (Tillberg et al., 2006). Percent carbon and nitrogen along with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaf tissue, seeds, and ants were analyzed in the Idaho Stable Isotopes Laboratory, Moscow, Idaho. Samples were analyzed

on a Finnigan-MAT, Delta + isotope ratio mass spectrometer (IRMS) using a CE Instrument's NC 2500 elemental analyzer to combust the samples. Instrument results are reported in delta ( $\delta$ ) notation on a per mil (‰) basis,  $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ , where  $R$  is the ratio of the heavy to the light isotope.

### 2.4. Statistical analysis

Linear regression analysis (R version 3.0.2; 2013-09-25; The R Foundation for Statistical Computing) was used to test dependent variables (Table A2) against the independent variable, "distance from Cabazon".  $P$  values were considered statistically significant when  $P < 0.05$ .

## 3. Results

### 3.1. Gradients

Atmospheric nitrogen deposition was significantly greater on the western side of the gradient ( $R^2 = 0.714$ ;  $P < 0.0001$ ) (Fig. 1a). Soil  $\text{NH}_4^+$  decreased significantly from west to east along the gradient ( $R^2 = 0.375$ ;  $P < 0.0001$ ) (Fig. 1b). However  $\text{NO}_3^-$  did not significantly change across the study area ( $R^2 = 0.050$ ;  $P = 0.103$ ), and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  values diverged along the west-east gradient (Fig. 1b). There was high variability in the soil nitrogen values, including several samples that had very low nitrate or ammonium (Fig. 1b). Although not significant, there was a suggestion of a decline in atmospheric  $[\text{CO}_2]$  decreased from west to east

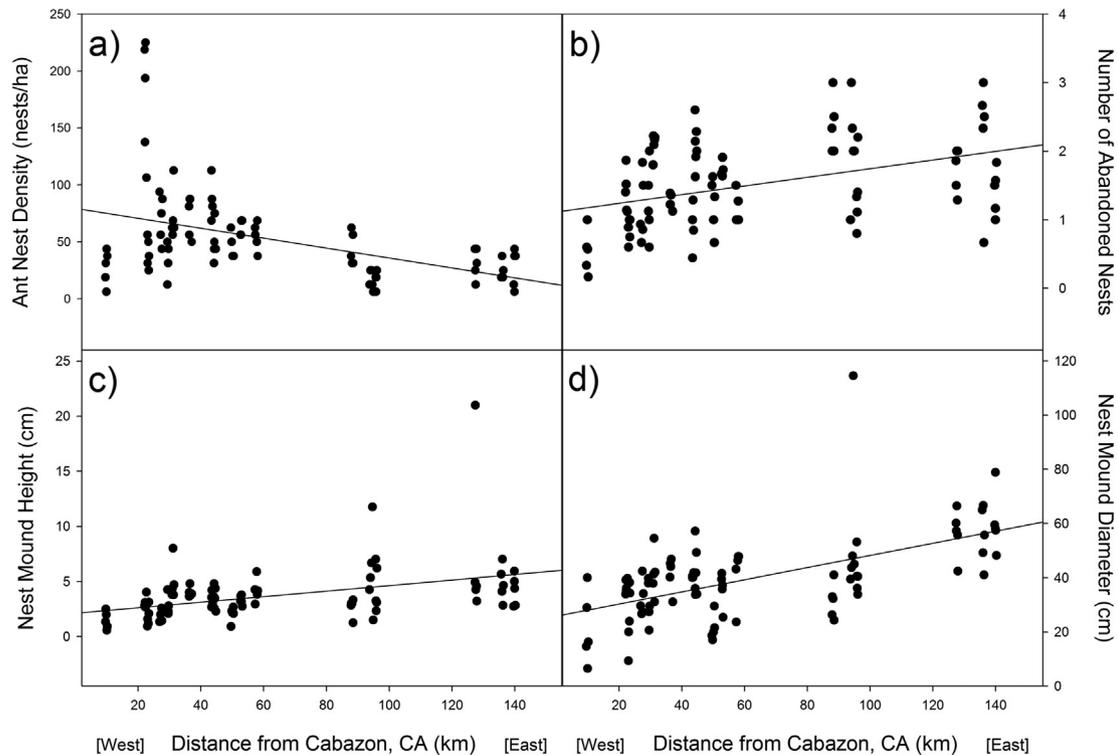


Fig. 2. *Messor pergandei* nest density (a), number of abandoned nests (b), nest mound diameter (c) and height (d) across the gradient, west to east from Cabazon, CA.

( $P = 0.185$ ) (Fig. 1c).  $\delta^{13}\text{C}$  values for background atmospheric  $\text{CO}_2$  did not significantly change across the nitrogen gradient from west to east ( $R^2 = 0.007$ ;  $P = 0.735$ ). Precipitation was higher closer to Cabazon (west) than on the east side of the study area ( $R^2 = 0.667$ ;  $P < 0.0001$ ) (Fig. 1d). The nitrogen deposition and precipitation gradients were significantly correlated ( $R^2 = 0.661$ ;  $P < 0.0001$ ).

### 3.2. Shrub and ant nest surveys

There were no significant differences in *L. tridentata* or *A. dumosa* shrub densities (both species ranged from under 100 to almost 1000 plants/ha) along the gradient (Table A2). *Larrea tridentata* produced more fruits and seeds on the west side of the gradient, ranging from over 30 fruits/seeds in areas of high nitrogen to under 10 fruits/seeds on the low nitrogen side of the gradient (Table A2). We were unable to obtain *A. dumosa* fruit and seed counts at all sites since some shrubs had dropped their fruits and seeds before sampling began. At eight sites, there were higher numbers of *A. dumosa* fruits and seeds at sites with high nitrogen deposition along the gradient ( $R^2 = 0.80$ ;  $P = 0.003$ ).

*Messor pergandei* nest density was significantly greater closer to Cabazon than on the east side of the gradient (Fig. 2a; Table A2). Abandoned nests surrounding the active colony were significantly denser at sites further from Cabazon (Fig. 2b; Table A2). Nest mounds were wider and taller at sites nearer to Cabazon (Fig. 2c and d; Table A2).

### 3.3. Percent carbon and nitrogen and isotopic composition

As nitrogen decreased across the gradient,  $\delta^{13}\text{C}$  values were more enriched. *Ambrosia dumosa* and *L. tridentata* leaf  $\delta^{13}\text{C}$  values significantly increased across the nitrogen gradient from west to east (Fig. 3a; Table A2). Seeds from *A. dumosa*, *L. tridentata*, and annuals also had significant patterns of  $\delta^{13}\text{C}$  enrichment across the

gradient from west to east (Fig. 3b; Table A2). In addition, the  $\delta^{13}\text{C}$  of ants became significantly more enriched from west to east (Fig. 3c; Table A2).

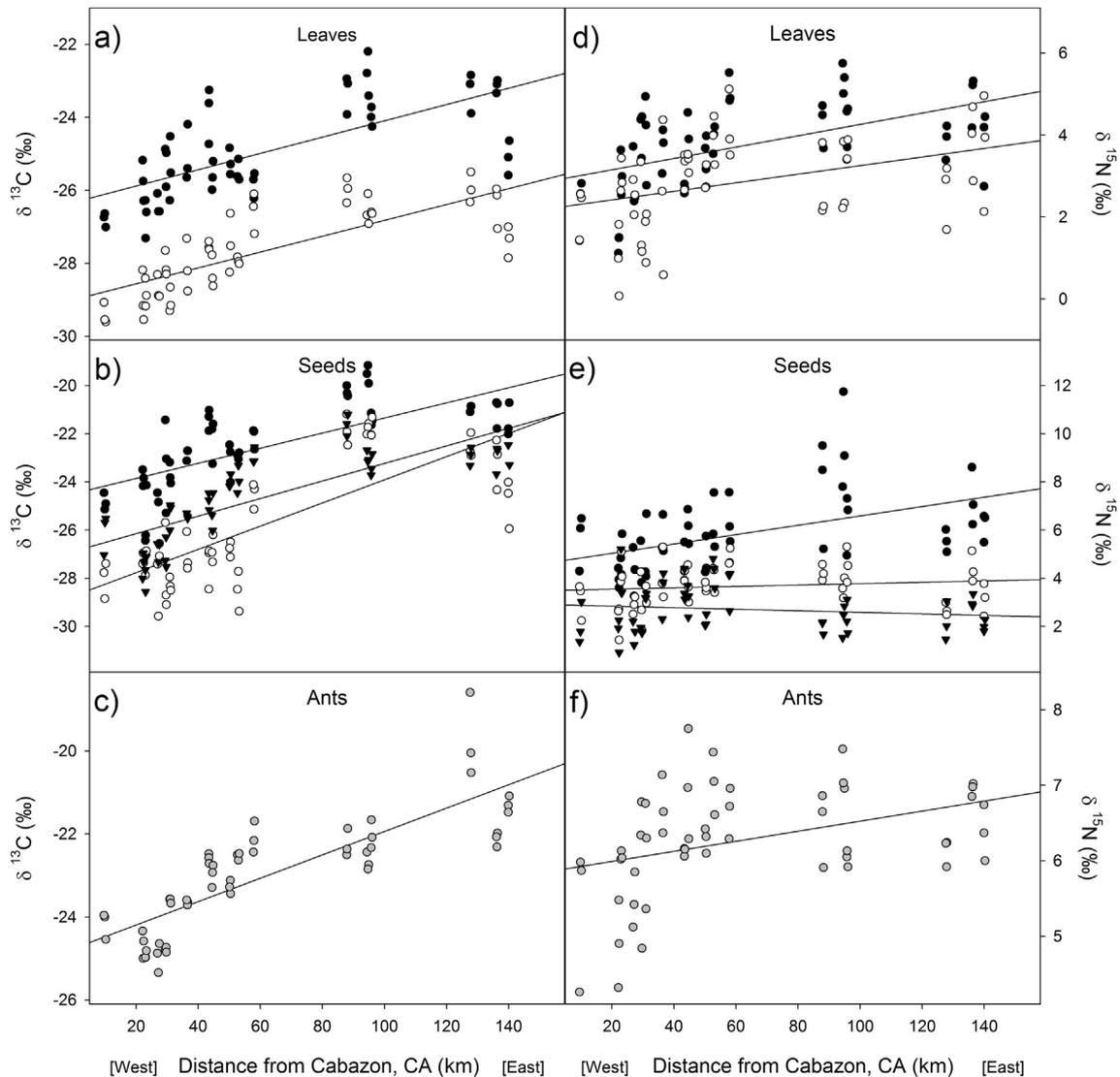
*Ambrosia dumosa* and *L. tridentata* leaf  $\delta^{15}\text{N}$  values were significantly more enriched further from Cabazon (Fig. 3d; Table A2). *Larrea tridentata* seed  $\delta^{15}\text{N}$  was also more enriched further from Cabazon (Fig. 3e; Table A2). *Ambrosia dumosa* and annual seed  $\delta^{15}\text{N}$  revealed no significant trends across the gradient (Fig. 3e; Table A2). *Messor pergandei* were more  $\delta^{15}\text{N}$ -enriched on the low nitrogen end of the gradient compared to high nitrogen deposition sites (Fig. 3f; Table A2).

Nitrogen deposition had little effect on percent carbon within trophic levels. Percent carbon in *L. tridentata* leaves increased from west to east (Fig. 4a; Table A2). However, *A. dumosa* leaves were not significantly different along the gradient (Fig. 4a; Table A2). Percent carbon in *L. tridentata* seeds decreased across the gradient from west to east (Fig. 4b; Table A2). *Ambrosia dumosa* and annual seeds were not significantly different along the gradient (Fig. 4b; Table A2). There were no significant differences in percent carbon in ants across the gradient (Fig. 4c; Table A2).

Patterns of percent nitrogen were somewhat similar to percent carbon. Percent nitrogen in *A. dumosa* and *L. tridentata* leaves were not significantly different across the gradient (Fig. 4d; Table A2). As with percent carbon, percent nitrogen in seeds of *A. dumosa* and annual plants were similar from west to east (Fig. 4e; Table A2). However, percent nitrogen in *L. tridentata* seeds significantly decreased from west to east, as shown with percent carbon in *L. tridentata* seed (Fig. 4e; Table A2). Percent nitrogen in ants was also not significantly different across the gradient (Fig. 4f; Table A2).

## 4. Discussion

*Larrea tridentata* and *A. dumosa* shrub densities were unchanged along the gradient, but there were more shrub fruits



**Fig. 3.**  $\delta^{13}\text{C}$  (left panel) values for *Larrea tridentata* (LATR) (open circles) and *Ambrosia dumosa* (AMDU) (black circles) leaves; *Larrea*, *Ambrosia* and annual (black triangles) plant seeds; and ants (gray circles) across the gradient, west to east from Cabazon, CA.  $\delta^{15}\text{N}$  (right panel) values for *Larrea tridentata* (LATR) (open circles) and *Ambrosia dumosa* (AMDU) (black circles) leaves; *Larrea*, *Ambrosia* and annual (black triangles) plant seeds; and ants (gray circles) across the gradient, west to east from Cabazon, CA.

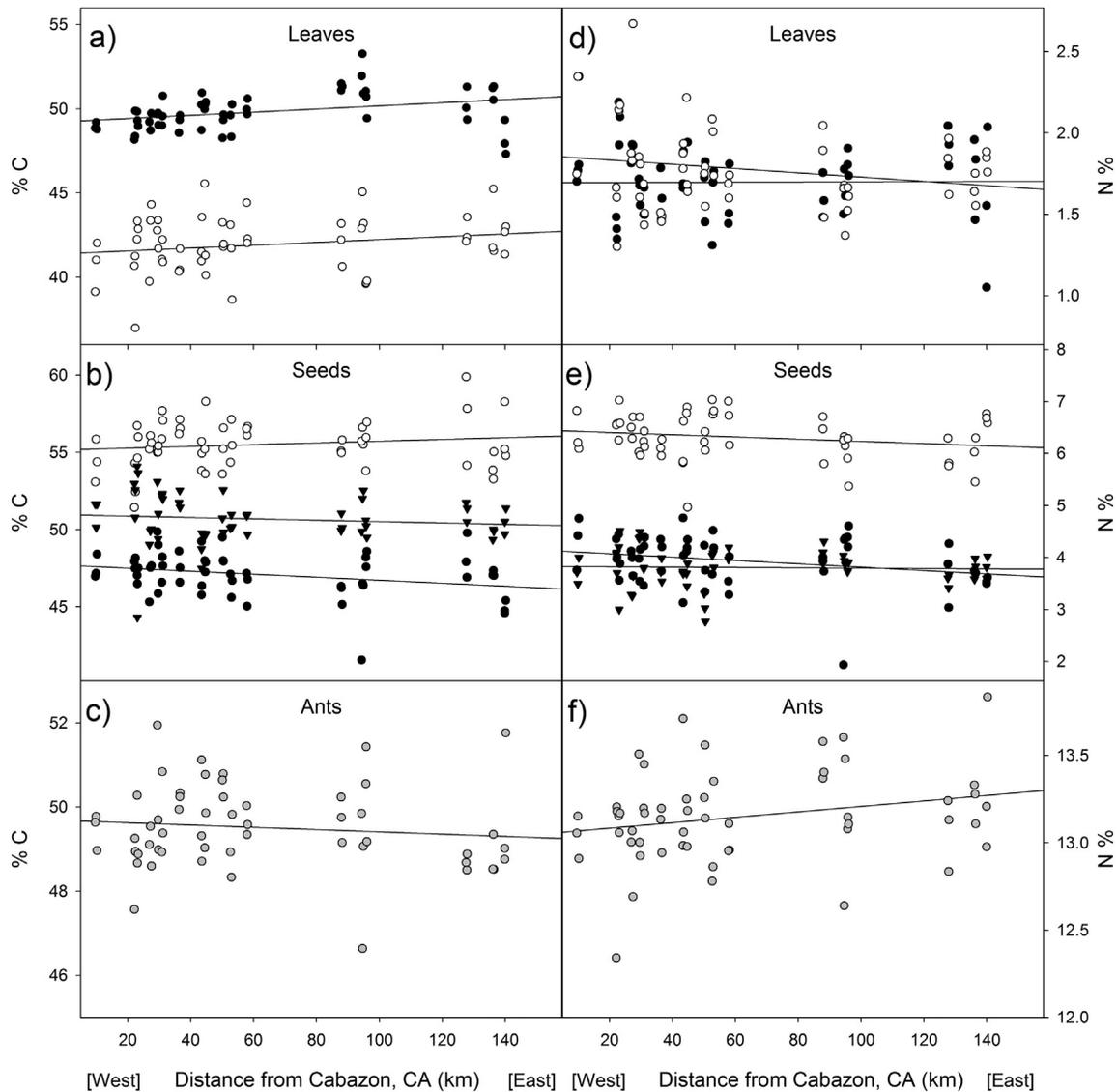
and seeds on the west side of the gradient with higher nitrogen deposition. *Messor pergandei* nest densities and mound size also varied across the gradient. In addition,  $\delta^{13}\text{C}$  and several of the  $\delta^{15}\text{N}$  values of the plants and ants were different from one end of the gradient to the other. These results suggest that as one travels east from Cabazon, CA, ecological changes are occurring, possibly due to nitrogen deposition. The nitrogen deposition and precipitation gradients were remarkably similar making it difficult to determine their individual contributions to the found patterns.

#### 4.1. Ant nest and shrub characteristics

Although shrub density did not vary along the nitrogen deposition gradient, *L. tridentata* seed production was greater in high nitrogen deposition and precipitation areas. In addition, based on analysis and field observations, *A. dumosa* seed production patterns indicated that nitrogen deposition and precipitation increased fruit and seed production. Considering desert shrubs are long-lived and

slow-growing with few recruitment events, shrub densities may not respond as readily to changes in nitrogen availability as reproduction. Thus, any changes in shrub densities may take longer to realize.

We predicted that nitrogen deposition would have positive effects on granivores, promoting plant resources and resulting in a bottom-up trophic cascade. Consistent with this prediction, *Messor pergandei* nest densities were greater in areas with higher nitrogen deposition, which may be a function of the increased seed production found in these areas. Harvester ants will travel long distances (tens of metres) to collect seeds, but abundant nearby seeds would promote establishment of new colonies (Gordon, 1995; Johnson, 2001; Went et al., 1972). In addition, precipitation may also explain the colony patterns we observed since soil moisture influences the success of harvester ant founder queens (Johnson, 2001). Although *M. pergandei* is adapted for desert environments, desiccation is a primary cause of early colony failure (Rissing and Pollock, 1985). Thus, in addition to high nitrogen, higher precipitation on the west end of the gradient



**Fig. 4.** Percent C (left panel) values for *Larrea tridentata* (LATR) (open circles) and *Ambrosia dumosa* (AMDU) (black circles) leaves; *Larrea*, *Ambrosia* and annual (black triangles) plant seeds; and ants (gray circles) across the gradient, west to east from Cabazon, CA. Percent N (right panel) values for *Larrea tridentata* (LATR) (open circles) and *Ambrosia dumosa* (AMDU) leaves (black circles); *Larrea*, *Ambrosia*, and annual plant seeds (black triangles); and ants (gray circles) across the gradient, west to east from Cabazon, CA.

may result in greater nest success and also contribute to the higher nest densities.

Once established, harvester ant colonies have a lifespan of 15–20 years with reproductive maturity at about five years; we found a greater abundance of smaller nests (and thus presumably younger nests) in the high nitrogen and precipitation areas. Although the effects of nitrogen and precipitation are somewhat confounded, we suspect that the effects of nitrogen on nest colonization may be relatively new, considering the recent phenomenon of anthropogenic nitrogen deposition as compared with the longevity of the precipitation gradient. The smaller nest sizes in areas of high nitrogen deposition and precipitation may also result from intraspecific competition. Reduced resources in low nitrogen areas may limit the number of colonies that survive initial colonization and allow the remaining colonies to grow large in size. In contrast, abundant seed sources and higher soil moisture under higher nitrogen deposition and precipitation may increase survivorship of colonies during establishment but lead to

greater competition among established colonies constraining colony enlargement.

#### 4.2. Carbon and nitrogen patterns

Carbon and nitrogen isotope patterns in ants and plants along the nitrogen deposition and precipitation gradient suggest that nitrogen assimilation alone does not explain increases in plant production and ant nests and that precipitation is highly influential in determining these patterns. The patterns of  $\delta^{13}\text{C}$  enrichment across the nitrogen and precipitation gradients (from high resources to low) indicate that plants were water stressed at the low precipitation end of the gradient. Plants discriminate against the heavier carbon isotope,  $^{13}\text{C}$ , during photosynthesis (Dawson et al., 2002; Farquhar et al., 1989). However, plants in drought conditions decrease stomatal opening, resulting in relatively  $^{13}\text{C}$ -enriched  $\text{CO}_2$  being used during photosynthesis (Farquhar et al., 1989). The increasing  $\delta^{13}\text{C}$  enrichment with increasing water

stress along the nitrogen deposition gradient is consistent with this mechanism and with similar patterns observed in vegetation in the Mojave Desert (Newingham et al., 2013) and in conifer seedlings in temperate climates (Dawson et al., 2002; Pinto et al., 2012).

Independent of water stress, high nitrogen concentrations may also increase  $\delta^{13}\text{C}$  as a consequence of increased biochemical demand for  $\text{CO}_2$  within the leaf (Dursma and Marshall, 2006). However, such a nitrogen response would be in the opposite direction from what we observed. If the nitrogen response dominated photosynthetic gas-exchange, then  $\delta^{13}\text{C}$  would have been highest at the urban end of the gradient, which is contrary to our findings. Although there are many processes that occur between photosynthetic traits and ant responses, our  $\delta^{13}\text{C}$  results reinforce the importance of the precipitation gradient. Alternatively, the differences in  $\delta^{13}\text{C}$  values across the gradient may also signify different sources of carbon.  $\text{CO}_2$  from fossil fuel combustion is more  $\delta^{13}\text{C}$  depleted than the  $\delta^{13}\text{C}$  in atmospheric  $\text{CO}_2$  near emission sources (Djuricin et al., 2010). Automobile exhaust is higher on the west side of the gradient; therefore, depletion of the  $\delta^{13}\text{C}$  values on the west side of the gradient suggests plants may be sourcing carbon from fossil fuel combustion.

The enrichment of  $\delta^{15}\text{N}$  in leaves and seeds with decreasing nitrogen contrasts with results in more mesic hardwood and conifer forests, which revealed a positive correlation with  $\delta^{15}\text{N}$  and nitrogen deposition (Pardo et al., 2006; Talhelm et al., 2012). The increase in  $\delta^{15}\text{N}$  in these other ecosystems may be due to nitrogen saturation and increased nitrification (Pardo et al., 2006), whereas nitrogen saturation and accelerated nitrification may not have occurred in areas of high nitrogen deposition in this desert system. We propose that although nitrogen deposition is increasing atmospheric nitrogen on the west side of the gradient, increased atmospheric deposition may not result in increased soil and plant nitrogen.

Differences in  $\delta^{15}\text{N}$  could also be due to specific source  $\delta^{15}\text{N}$  values along the gradient (Bell, 2012; Pardo et al., 2006). Assuming that the air currents that brought the polluted carbon also delivered the nitrogen, it is possible that atmospheric nitrogen from the urban areas is eventually being taken up by harvester ants. The differences in  $\delta^{15}\text{N}$  values for leaves, seeds, and ants across the gradient imply that the sources of nitrogen or the nitrification rate differs across the nitrogen deposition gradient (Pardo et al., 2006).

If sites along the gradient are equally divided into three nitrogen depositional levels (high, medium, and low N), differences in  $\delta^{15}\text{N}$  values among trophic levels are evident. Ant  $\delta^{15}\text{N}$  values were about three per mille more enriched than the  $\delta^{15}\text{N}$  values of the annual seeds in each nitrogen deposition level. This enrichment is consistent with the expected  $\Delta \delta^{15}\text{N}$  of three per mille with each trophic level beginning with first level herbivores (DeNiro and Epstein, 1981; McCutchan et al., 2003). Although several studies have shown that herbivores in desert ecosystems have elevated  $\delta^{15}\text{N}$  values due to physiological stress from temperature and aridity, Hartman (2011) demonstrated that  $\delta^{15}\text{N}$  values of two desert herbivores likely reflected the  $\Delta \delta^{15}\text{N}$  of three per mille between the diet and animal tissues rather than environmental stress. Our study corroborates this finding, as the ants were separated from the annual seeds with  $\Delta \delta^{15}\text{N}$  of three per mille between the two trophic levels. In contrast, *L. tridentata* seed  $\delta^{15}\text{N}$  values were nearly identical to the ant  $\delta^{15}\text{N}$  values in the high and medium nitrogen levels and were more enriched than the ants in the low nitrogen level. This suggests that *L. tridentata* seeds are not a primary food source for *M. pergandei* across the nitrogen gradient.

The similarities in percent nitrogen for plants and ants across the nitrogen deposition gradient indicate that homeostatic regulation of carbon and nitrogen by organisms may be obscuring patterns across these environmental gradients in the southern California deserts (Sterner and Elser, 2002). Although changes in plant community and production due to nitrogen deposition have been reported in aquatic and more mesic ecosystems (Jones and Power, 2012), other studies in *L. tridentata*/*A. dumosa* communities have found small to no changes in percent nitrogen of vegetation in response to nitrogen deposition (Hall et al., 2011; Newingham et al., 2012; Smith et al., 2009) or found the combination of nitrogen and precipitation was required to induce change (Rao and Allen, 2010).

The effects of chronic nitrogen deposition are likely dependent upon precipitation and thus soil moisture availability, as inorganic nitrogen that is deposited on the desert soil surface lies inert until it rains. Longer-lived organisms, such as *Larrea* and the ants, may benefit from the high levels of resources on the west side of the gradient, which allow them to store available resources for times of adverse conditions. The shorter-lived *Ambrosia* and annual plants may also benefit, as they may quickly capitalize on sporadic nitrogen availability during and after rain events. Considering precipitation is highly variable from year to year in these warm deserts, further research is necessary to disentangle the effects of nitrogen and precipitation.

## 5. Conclusion

Altered nitrogen inputs into this desert ecosystem were predicted to have cascading effects through trophic levels. We found higher seed production, higher ant nest densities, and smaller nests in high nitrogen areas suggesting that nitrogen deposition may be increasing food supplies for harvester ants and promoting colonization of new nests; however, these ecosystem responses are also likely influenced by the precipitation gradient. In addition, variation in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values along the nitrogen deposition gradient reveal sources of carbon and nitrogen across the gradient are different and evidently influenced by anthropogenic activities. Our results suggest that two trophic levels (annual plants and harvester ants) are taking up anthropogenic nitrogen, which likely affects vegetation and harvester ant communities. Further work should investigate the interplays between nitrogen and precipitation along this gradient. Harvester ants are one of the primary ecosystem engineers in desert ecosystems and their ability to move seed rivals that of any desert animal. Thus, it is important to understand how these granivores adjust to global changes and the resultant cascading effects on desert plant biodiversity and communities.

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## Appendix

Table A1

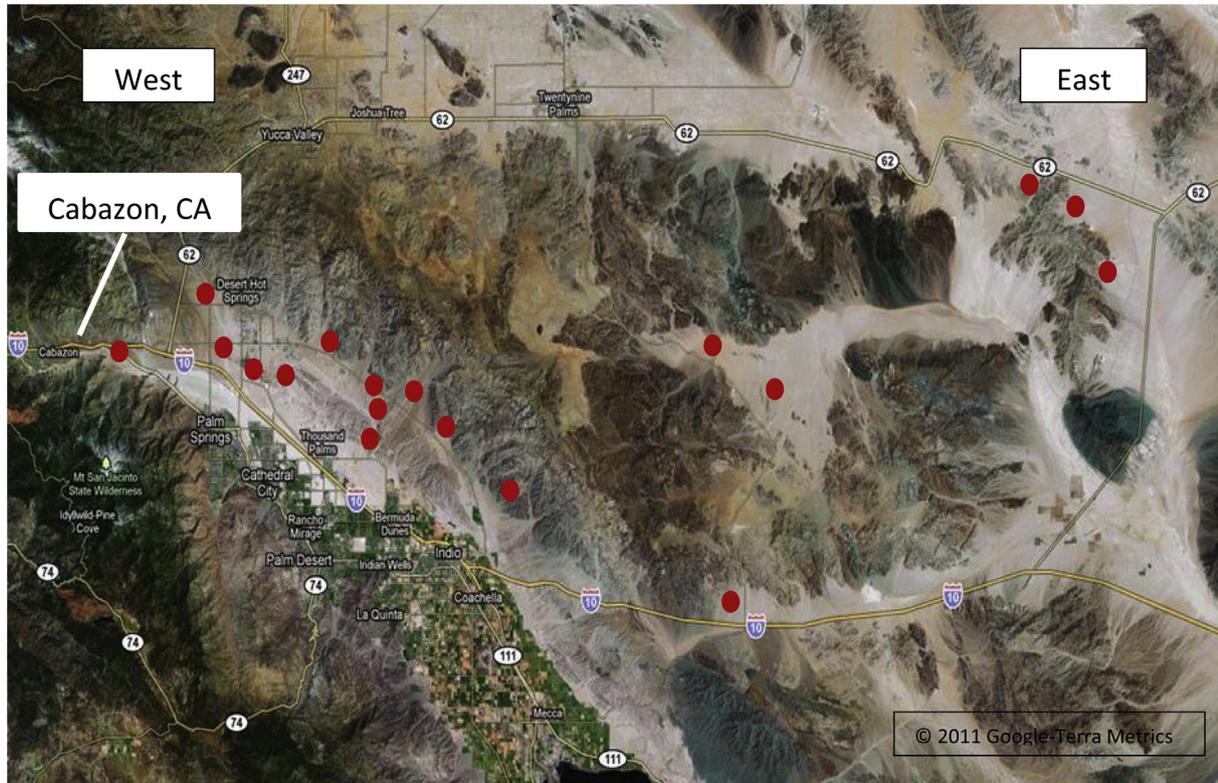
Site descriptions across the nitrogen deposition study area with distance from Cabazon, site type, soil texture, and elevation.

Site	Distance from Cabazon, CA (km)	Site type	Soil texture	Elevation (m)
Snow Creek	10	Sandy wash	Sand	369
N. Indian Canyon	22	Basin	Loamy sand	454
Windy Meadows	23	Basin	Sand	234
Palm Drive	27	Basin	Loamy sand	180
Mtn. View/20th	29	Basin	Sand	240
Long Canyon	31	Bajada	Loamy sand	392
Wide Canyon	37	Bajada	Loamy sand	523
Willis Palms	44	Bajada	Sand	91
TNC 1	44	Bajada	Loamy sand	232
Fan Hill	50	Bajada	Loamy sand	533
Indio Hills	53	Bajada	Loamy sand	405
Berdoo Canyon	58	Bajada	Loamy sand	280
Magic Circle	88	Basin	Loamy sand	540
JT South	94	Basin	Loamy sand	528
Pinto Basin	96	Basin	Loamy sand	715
Cadiz Valley	128	Bajada	Loamy sand	542
Coxcombcell	136	Bajada	Loamy sand	375
Aqueduct	140	Bajada	Loamy sand	360

Table A2

 $R^2$  and  $P$  values for distance from Cabazon, CA as the independent variable for all listed dependent variables for *M. pergandei* (ANT), *L. tridentata* (LATR), *A. dumosa* (AMDU) and selected annual plants. Significant ( $P < 0.05$ ) values are bolded.

Dependent variable	$R^2$ value	$P$ value
<i>Messor pergandei</i> nest density	<b>0.197</b>	<b>&lt;0.0001</b>
Abandoned ant nest density	<b>0.165</b>	<b>&lt;0.0001</b>
Ant nest mound diameter	<b>0.35</b>	<b>&lt;0.0001</b>
Ant nest mound height	<b>0.164</b>	<b>&lt;0.0001</b>
<i>Larrea tridentata</i> fruits	<b>0.422</b>	<b>&lt;0.0001</b>
<i>Ambrosia dumosa</i> shrub density	0.017	0.341
<i>Larrea tridentata</i> shrub density	0.026	0.246
<i>Ambrosia dumosa</i> leaf $^{13}C$	<b>0.594</b>	<b>&lt;0.0001</b>
<i>Larrea tridentata</i> leaf $^{13}C$	<b>0.493</b>	<b>&lt;0.0001</b>
<i>Ambrosia dumosa</i> seed $^{13}C$	<b>0.599</b>	<b>&lt;0.0001</b>
<i>Larrea tridentata</i> seed $^{13}C$	<b>0.521</b>	<b>&lt;0.0001</b>
Annual Plants seed $^{13}C$	<b>0.625</b>	<b>&lt;0.0001</b>
<i>Messor pergandei</i> $^{13}C$	<b>0.681</b>	<b>&lt;0.0001</b>
<i>Ambrosia dumosa</i> leaf $^{15}N$	<b>0.149</b>	<b>0.004</b>
<i>Larrea tridentata</i> leaf $^{15}N$	<b>0.265</b>	<b>&lt;0.0001</b>
<i>Ambrosia dumosa</i> seed $^{15}N$	0.017	0.349
<i>Larrea tridentata</i> seed $^{15}N$	<b>0.233</b>	<b>0.0002</b>
Annual plants seed $^{15}N$	0.018	0.334
<i>Messor pergandei</i> $^{15}N$	<b>0.138</b>	<b>0.006</b>
<i>Ambrosia dumosa</i> leaf %C	0.041	0.14
<i>Larrea tridentata</i> leaf %C	<b>0.116</b>	<b>0.011</b>
<i>Ambrosia dumosa</i> seed %C	0.022	0.285
<i>Larrea tridentata</i> Seed %C	<b>0.074</b>	<b>0.047</b>
Annual plants seed %C	0.013	0.41
<i>Messor pergandei</i> %C	0.013	0.417
<i>Ambrosia dumosa</i> leaf %N	0.442	0.139
<i>Larrea tridentata</i> leaf %N	<0.0001	0.949
<i>Ambrosia dumosa</i> seed %N	0.042	0.139
<i>Larrea tridentata</i> seed %N	<b>0.073</b>	<b>0.048</b>
Annual plants seed %N	0.001	0.788
<i>Messor pergandei</i> %N	0.057	0.083



**Figure A1.** Map of study sites across the N deposition gradient in southern California. Sites (circles) were measured as the distance from Cabazon.

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