

Does Tall Oatgrass Accelerate Soil Nitrogen Cycling in Boulder Open Space and Mountain Parks Land?

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Executive Summary

This study addressed whether Tall Oatgrass (*Arrhenatherum elatius* subsp. *elatius*), an invasive perennial C3 grass that threatens the persistence of native tallgrass prairie ecosystems in City of Boulder Open Space and Mountain Parks (OSMP) lands, accelerates soil nitrogen (N) cycling. Globally, many invasive plant species alter their soil environment, often creating conditions that favor their growth over natives. In addition, C3 grasses are known to drive a more open—or leaky—N cycle compared with C4-dominated systems, increasing the movement of N from soils into the atmosphere or aquatic systems, as well as the potential for shifts in plant community composition. At three sites that typify locations and patterns of *A. elatius* invasion in Boulder OSMP lands, rates of net N mineralization and nitrification (the processes that transform organic N to more plant-accessible inorganic N), inorganic N pools, soil properties, as well as aboveground biomass and carbon-to-nitrogen (C:N) ratios were measured over the course of a year; measurements of net N cycling rates were targeted during key phenological periods including Mar-Apr 2020 (snowmelt/green-up), June-July 2020 (peak biomass), and Sept-Oct 2020 (plant senescence, seasonal transition) to capture temporal differences in soil N dynamics. Results indicate that net N mineralization was higher in invaded conditions at two of three sites (significant interaction of invasion level and site; $p < 0.05$) during Summer 2020. Net nitrification was significantly higher in invaded conditions regardless of site during Summer 2020. Measurements of variables related to soil and vegetation did not demonstrate a clear relationship associated with trends in net N cycling rates. However, aboveground biomass was greater in invaded than uninvaded areas, indicating that *A. elatius* may have additional effects on ecosystem processes, including rates of decomposition. Next steps in this research are to consider differences in soil microbial communities between invaded and uninvaded areas and to

determine whether native C4 prairie species of interest (e.g., *Andropogon gerardii*) can be restored in areas where *A. elatius* has persisted. Related to management of *A. elatius* by Boulder OSMP, the following recommendations should be considered:

- Not all areas invaded by *A. elatius* function the same; different management tactics related to *A. elatius* control must be considered across sites;
- Further research into the details of how the soil N cycle changes under *A. elatius* would be important to advance knowledge about how this biogeochemical cycle is changing in the Colorado Front Range, but are probably not necessary to inform management actions;
- Next steps are to determine whether or not native plant species can be restored in areas previously inhabited by *A. elatius* and where soil N enrichment exists as a result of the invasive species' presence.

1 **Abstract**

2 Decades of research have documented changes to nitrogen (N) cycling in the alpine zone of the
3 Colorado Rockies, yet fewer efforts have occurred in lower elevations close to anthropogenic N
4 sources and human activities in the Denver-Boulder area. This study focuses on this understudied
5 elevation zone and takes a first look at the relationships between a persistent invasive plant
6 species, Tall Oatgrass (*Arrhenatherum elatius*), and soil N cycling. At three locations managed
7 by City of Boulder Open Space and Mountain Parks (OSMP), this effort examined differences in
8 soil N transformations, inorganic N pools, soil moisture, aboveground biomass, and soil and
9 vegetation C:N ratios across plots containing 40-80% (invaded) and 0% (uninvaded) cover of *A.*
10 *elatius*. Principle findings include higher rates of net N mineralization and net nitrification in
11 invaded compared with uninvaded plots at two of the three sites (significant interaction of
12 invasion x site, $p < 0.05$). Highest net N cycling rates were observed during summer (peak
13 biomass) and lowest rates—indicating N immobilization at most uninvaded locations—occurred
14 during fall. Although patterns in possible explanatory variables were not consistent by invasion
15 level or across sites, generally, aboveground biomass amounts were greater in invaded than
16 uninvaded areas, which may have feedbacks to ecosystem processes (e.g., decomposition rates,
17 growth and success rates of other plant species). These findings suggest a slightly accelerated
18 soil N cycle in *A. elatius*-invaded areas, but that the factors related to invasion and N cycling
19 differ by site. Recommendations for Boulder OSMP include determining whether native plant
20 species can be restored to inorganic N-enriched soils once *A. elatius* is removed. More broadly,
21 this research contributes to understanding how the N cycle is changing under the increasing
22 pressures of human activities in the Denver-Boulder area.

23 **Keywords:** *Arrenatherum elatius*, nitrification, net N mineralization, tallgrass prairie, grassland
24 restoration

25 **Introduction**

26 Fossil fuel combustion, land use change, and use of synthetic fertilizers have more than
27 doubled the amount of reactive nitrogen (N) cycling through air, land, and water systems since
28 pre-Industrial times (Davidson et al. 2011, Gruber and Galloway 2008). It is well known that
29 elevated atmospheric N deposition is a threat to ecosystems worldwide, causing lower plant
30 diversity (Field et al. 2014), accelerated soil biogeochemical cycling and acidification (Matson et
31 al. 2002), and eutrophication of surface waters (Baron et al. 2013). The links between human
32 sources of N and their ecosystem effects have been described as “The Nitrogen Cascade” by
33 Galloway and colleagues (Galloway et al. 2003). In the Colorado Front Range and Rocky
34 Mountains, ecosystems from the plains to the alpine are impacted by human activities in the
35 nearby developed and agricultural areas that increase atmospheric N deposition two-to-three-fold
36 on the eastern compared with the less populated western slope (e.g., Wetherbee et al. 2019,
37 Baron et al. 2000). For the very sensitive ecosystems of this region, such increases can be
38 destabilizing.

39 While several decades of research have documented how N cycling is changing alpine
40 ecosystems in the Rocky Mountains, there has been markedly less research at lower elevations in
41 the Colorado Front Range – that is, foothills and prairie ecosystems—many of which are
42 managed by The City of Boulder Open Space and Mountain Parks (OSMP). These areas receive
43 significantly higher atmospheric deposition of ammonium-N than the alpine, likely from
44 agricultural sources (Crawford et al. 2020). It is widely known that high soil N environments can
45 give invasive plants a competitive advantage (Dukes and Mooney 1999). Often, a positive
46 feedback ensues: the invasive continues to enrich the soil inorganic N pool, perpetuating
47 conditions that favor its success, changing plant species composition and N cycling (Bobbink et

48 al. 2010). Thus, increases in atmospheric N deposition to Boulder OSMP lands may threaten the
49 persistence of native plant communities that support a diversity of invertebrate and vertebrate
50 species and are valued for recreation.

51 Tall oatgrass (*Arrhenatherum elatius* subsp. *elatius*) is an invasive perennial grass present
52 in the foothills and prairie lands managed by Boulder OSMP. A principal concern of Boulder
53 OSMP managers is that elevated atmospheric N deposition may encourage growth of *A. elatius*,
54 and the invasive plant may change ecosystem functioning, including soil nutrient cycling,
55 limiting the ability of natives to return to and persist on OSMP lands (EnviroPlan Partners 2018).
56 Originally introduced in the mid-19th century by ranchers, spread of *A. elatius* was likely
57 suppressed by grazing, but with cessation of grazing around the 1960s, as well as fire
58 suppression, it spread easily (EnvironPlan Partners 2018). During the last decade, Boulder
59 OSMP has been evaluating several management approaches to contain *A. elatius*, including
60 prescribed fire, which, independently, can increase soil inorganic N pools (DeLuca and Zouhar
61 2000), pesticide applications, weed-whipping, and grazing. The goal of our study was to
62 determine the relationship between *A. elatius* and an altered soil N cycle. Specifically, we
63 addressed the question, Does invasion by *A. elatius* accelerate soil N cycling in grasslands? In
64 addressing this knowledge gap, we sought to inform understanding of how the invasive plant
65 affects ecosystem functioning; this was a first step to inform future studies that address the
66 interactions among *A. elatius*, soil N cycling, and management practices. More broadly, it can
67 also provide a foundation for conducting process-based study of the relationships among
68 atmospheric N deposition, soil N cycling, and ecosystem functioning along the entire elevation
69 gradient, from the plains to the alpine.

70 We established study plots across three sites in grassland and woodland areas of the
71 Colorado Front Range that capture the types of settings invaded by *A. elatius*. Within 40-80% *A.*
72 *elatius* invaded (referred to as “invaded”) and *A. elatius* uninvaded (referred to as “uninvaded”)
73 plots at each site (Table 1), we compared measurements of net N mineralization (i.e.,
74 microbially-mediated transformation of more stable soil organic N to plant available forms,
75 ammonium and nitrate) and net nitrification (i.e., microbially-mediated transformation of
76 ammonium to nitrate) rates, as well as inorganic N pools, soil moisture, soil and plant tissue C:N,
77 and aboveground biomass. Our study was designed to test the hypothesis that with invasion of *A.*
78 *elatius*, soil N processing rates and inorganic N pools will increase above those of uninvaded
79 areas, indicating an altered N cycle. We measured the accompanying variables in order to assess
80 whether they might explain differences between invaded and uninvaded areas. Our approach
81 aligns with previous research by Ehrenfeld et al. (2003) showing higher biomass (thatch
82 production) and tissue turnover driving accelerated N cycling in non-native, invasive plant
83 communities. Our approach however, unlike that of Ehrenfeld and colleagues, focuses on *A.*
84 *elatius*.

85 **Methods**

86 *Study Area*

87 We conducted this research at three sites in the foothills of the Colorado Front Range, all
88 managed by Boulder OSMP (Figure 1). The three sites were chosen for their representativeness
89 with respect to the pattern of invasion by *A. elatius*: on Shanahan Ridge in a woodland area with
90 dense patches (~5-10 m) of *A. elatius* invasion interspersed with other grasses and trees; south of
91 the NCAR mesa where there are broad swaths of dense *A. elatius* invasion, the highest percent
92 cover observed in this study; and Coyote Canyon, a steep ravine with north- (invaded) and south-

93 facing (largely uninvaded) slopes. In part, we chose these locations to determine the general
94 differences in soil N cycling rates between *A. elatius* invaded and uninvaded areas across the
95 foothills area; however, we also recognized that there may be underlying differences in soil
96 moisture patterns and temperature across sites that would be worth considering and could
97 possibly explain variation in rates of soil N processes. Average monthly air temperature in the
98 Denver-Boulder Metropolitan area ranges from approximately 0 °C in winter to 21 °C in summer
99 and mean annual precipitation to the foothills of the Colorado Front Range is ~790-890 mm
100 (Wetherbee et al. 2019), typical of tallgrass prairie systems. Other important characteristics of
101 the sites are summarized in Table 1.

102 *Study Design*

103 At each of three study sites, we established three 2m x 2m plots within *A. elatius* invaded
104 and uninvaded areas, respectively ($n = 18$). The patchiness of *A. elatius* invasion precluded
105 random plot location. Thus, we chose plot locations based on the following criteria: invaded
106 plots had to be placed within patches of 40-80% *A. elatius* cover, and uninvaded plots had to be
107 located at least 2m from the nearest *A. elatius* individual, with 0% *A. elatius* cover within the
108 plot. At Shanahan and NCAR, the plots were located in uninvaded and invaded areas along a
109 common slope and aspect. At Coyote Canyon, plots were located along two transects parallel to
110 the slope.

111 *Field Sampling*

112 Each site was sampled three times during the study period (14 March 2020 – 16 October
113 2020) for soil N processing rates, inorganic N pools, and gravimetric soil moisture. We targeted
114 key phenological periods to make these measurements: Mar-Apr 2020 (snowmelt/green-up),

115 June-July 2020 (peak biomass), and Sept-Oct 2020 (plant senescence, seasonal transition). For
116 each sampling period, paired soil cores (3 cm diameter x 10 cm depth) were collected at each
117 plot and placed in plastic bags, one for immediate analysis of soil inorganic N species, and the
118 other returned to its borehole for incubation, following the in-field buried bag technique
119 described by Hart et al. (1994). Following incubation, soil cores were transported to the
120 laboratory for analysis in the same manner as the one subjected to immediate analysis (see
121 description below). Once during the study period, we collected soil cores for soil C:N, soil pH,
122 and bulk density (September 2019, March 2020, and October 2020, respectively).

123 In addition to soil measurements, we also sampled vegetation within each plot for
124 aboveground biomass, leaf tissue C:N ratios, and species presence. We collected samples from
125 2m x 2m quadrats (one per plot) in September 2019. Standing stems, as well as thatch cover
126 were clipped to the ground surface within the quadrat; it is important to note that these
127 collections occurred when *A. elatius* had senesced at the end of summer/early fall. In the
128 laboratory, aboveground biomass samples were dried at 60°C for 48 hr, then weighed to
129 determine dried biomass per unit area. After cutting and mixing each sample, a homogeneous
130 subsample of aboveground biomass tissue was pulverized with a mortar and pestle and analyzed
131 for total C and N, as described below. We estimated plant species cover within a 1 m² area of
132 each plot using visual cover and then identified the presence of all remaining species within the
133 remaining 2 m² plot. Dominant plant species were considered any species that had > 20% areal
134 cover.

135 *Laboratory Analysis*

136 Immediately upon return from the field, soil cores were hand-picked to remove roots and
137 rocks. Sub-samples of field-moist soil were extracted in 2M potassium chloride (KCl), shaken

138 for two hours, and then filtered using Whatman 1 filters, modified from Binkley et al. (1986) and
139 Hart et al. (1994). Extractants were analyzed for ammonium and nitrate by standard method
140 4500-NH₃H phenolate flow injection, detection limit of 0.005 mg NH₄⁺-N L⁻¹ and with a Lachat
141 QuikChem 8500 Flow Injection Autoanalyzer, detection limit of 0.004 mg NO₃⁻-N L⁻¹,
142 respectively. In addition, a subsample of each soil sample was analyzed for gravimetric soil
143 moisture (dried at 105°C for 48 hr) and subsamples of oven-dried (60°C for 48 hr) and ground
144 soil and vegetation tissue were analyzed for total C and N by combustion on a Thermo Finnigan
145 Flash EA 1112. All analyses were completed in the Arikaree Environmental Laboratory at
146 University of Colorado, Boulder.

147 *Data Analysis*

148 We calculated rates of net N mineralization and net nitrification by differencing the mass
149 of inorganic N (ammonium and nitrate for net N mineralization and nitrate for net nitrification)
150 in final and initial soil extracts and dividing by the incubation period to get the mass of N
151 produced per gram of dry soil per day for each of three sampling periods. Both rates and
152 inorganic N pools are reported on an areal basis using bulk density to scale. We used *R* (version
153 1.2.5033) and a linear model to analyze the relationships between measured variables and
154 invasion at each site. We analyzed net mineralization rates, net nitrification rates, biomass,
155 vegetation C:N, and soil C:N as functions of invasion for each site. Our data meet the linear
156 model assumptions of linearity, homoskedasticity, normality of residuals, and independence.
157 Calculated *p*-values were used to determine significance (< 0.05). As a supplement, we fit four
158 additional models to quantitatively compare mineralization and nitrification by invasion, site,
159 and their interaction, for the summer and fall season.

160 Results

161 We hypothesized that soils where *A. elatius* grows have accelerated rates of soil N
162 cycling processes – net N mineralization and nitrification – and inorganic N pools compared with
163 those areas where the invasive is not present. Analysis of the net N cycling rates broken out by
164 season revealed that the largest differences between invaded and uninvaded plots occurred
165 during the summer (June through July). Coyote Canyon and NCAR both had higher net N rates
166 in invaded than uninvaded plots, yet only the differences in net N mineralization at NCAR were
167 statistically significant per our first set of statistical models: $36.03 \pm 12.40 \text{ mg m}^{-2} \text{ day}^{-1}$
168 compared with $9.61 \pm 10.69 \text{ mg m}^{-2} \text{ day}^{-1}$ ($p < 0.05$; Figure 2a, Table 2). The supplemental
169 model, however, did reveal a statistically significant difference in net N mineralization rates at
170 Coyote Canyon ($p < 0.05$). The opposite pattern occurred at Shanahan Ridge: uninvaded areas
171 had on average higher net N cycling rates than those invaded by *A. elatius*. Average net N
172 mineralization rates in invaded plots were slightly negative, indicating net N immobilization
173 (Figure 2a).

174 Rates of net N cycling in the fall (September-October) also revealed compelling, yet very
175 different patterns than during the summer. Across sites, net N mineralization rates were lower
176 overall than during the summer (Figure 2b). During the fall, slightly higher average net N
177 mineralization rates occurred in invaded versus uninvaded plots at both Coyote Canyon
178 (compare 5.18 ± 8.90 versus $-0.95 \pm 14.99 \text{ mg N m}^{-2} \text{ day}^{-1}$) and NCAR (compare 12.55 ± 15.71
179 versus $-9.23 \pm 11.55 \text{ mg N m}^{-2} \text{ day}^{-1}$), however, the results were not statistically significant ($p >$
180 0.05 ; Table 2) and moved toward net N immobilization (values at or below $0 \text{ mg m}^{-2} \text{ day}^{-1}$;
181 Figure 2b). Similarly, net nitrification rates were lower overall across sites, compared with
182 values during the summer (Figures 3a and 3b). However, with the exception of uninvaded plots

183 at NCAR, average rates were $> 0 \text{ mg m}^{-2} \text{ day}^{-1}$; at all sites, invaded plots had higher average rates
184 of net nitrification than uninvaded plots, but results were not statistically significant ($p > 0.05$).

185 There was not a clear pattern across sites, invasion level, or seasons (summer and fall
186 sampling periods) for either soil ammonium or nitrate pools (Figures 4 and 5). Inorganic N pools
187 in invaded plots were higher on average than uninvaded plots at both NCAR and Shanahan
188 Ridge during the summer; at Shanahan Ridge, the difference was as much as 6-fold and three-
189 fold greater on average for ammonium and nitrate respectively, yet variable across field
190 replicates (Figures 4a and 5a). During the fall, NCAR exhibited the opposite result, with higher
191 soil ammonium and nitrate pools in uninvaded versus invaded plots by approximately two-fold
192 (Figures 4a and 5b). Coyote Canyon had higher soil ammonium N pools in uninvaded compared
193 with invaded areas (approximately two-fold difference) and approximately 50% more nitrate in
194 uninvaded than invaded plots during both summer and fall. Overall, the largest soil inorganic N
195 pools were observed at Shanahan Ridge across both seasons. However, none of these
196 comparisons were statistically significant, ($p > 0.05$).

197 Our aboveground biomass harvests revealed slightly higher biomass in invaded than
198 uninvaded plots (Figure 6). Of the three sites, only the differences were statistically significant at
199 Coyote Canyon: $1321.3 \pm 361.7 \text{ g m}^{-2}$ versus $575.8 \pm 294.4 \text{ g m}^{-2}$ ($p < 0.05$). Comparison of C:N
200 ratios of aboveground biomass in invaded versus uninvaded areas demonstrated that within sites,
201 there were differences between the two treatments, but the direction was not the same across
202 sites. For example, at the NCAR site, vegetation C:N was 51.2 ± 8.3 in invaded and 37.1 ± 5.8 in
203 uninvaded ($p = 0.07$; Table 1). However, at Shanahan Ridge, invaded plots had on average lower
204 C:N than uninvaded plots (compare 49.0 ± 3.0 and 58.9 ± 8.9), although the differences were not
205 statistically significant ($p > 0.05$). Measurement of the total N content of aboveground biomass

206 did reveal significantly higher concentrations in uninvaded than invaded plots at Coyote Canyon
207 ($p = 0.02$), while the opposite pattern occurred at Shanahan Ridge ($p = 0.04$).

208 We also measured three additional, potentially important explanatory variables for net N
209 cycling rates: soil C:N ratios, moisture, and pH. Bulk soil C:N ratios in invaded versus
210 uninvaded areas demonstrated that within sites, there were not significant differences between
211 the two treatments (Table 1). However, differences existed among sites. NCAR, for example,
212 had lower soil C:N in both treatment plots (~14) compared with Coyote Canyon (~17). Soil pH
213 and moisture values are summarized in Tables 1 and 3, respectively; statistically significant
214 differences did not occur between treatments or among sites. Across time periods, soil moisture
215 ranged from ~14-23%, with lowest soil moisture levels occurring during the summer sampling
216 period (Table 3); soil pH was 6.17-6.69 across treatments and sites without clear or statistically
217 significant differences between invaded and uninvaded areas (Table 1).

218 **Discussion**

219 *Soil N cycling in A. elatius-invaded and uninvaded areas*

220 This study addressed the question of whether *A. elatius*, an invasive grass in the Colorado
221 Front Range, alters the cycling of N, a nutrient that is often limiting to plant growth in terrestrial
222 ecosystems. In the Colorado Front Range, elevated atmospheric N deposition from
223 anthropogenic sources in the Denver-Boulder metropolitan area contribute double the N to
224 terrestrial ecosystems, compared with comparatively less developed areas in the region
225 (Wetherbee et al. 2019, Baron et al. 2000). We hypothesized that *A. elatius* may compound
226 regional soil N enrichment from atmospheric deposition by accelerating rates of the soil N
227 cycling processes, net N mineralization and net nitrification. Our rationale was that many
228 invasive plant species are successful in novel environments because of their ability to change the

229 soil environment—through N fixation (e.g., Kurokawa et al. 2010, Asner et al. 2008),
230 mechanisms of dispersal (e.g., Gallego-Fernández et al. 2020, Pergl et al. 2011), production of
231 N-rich tissues (e.g., Kurokawa et al. 2010), or biomass production (e.g., Aguilera et al. 2010).
232 While we know that *A. elatius* is not an N-fixing species, the degree to which it affects soil N
233 cycling, and the mechanisms by which it does so, have been largely unstudied. In particular, staff
234 at Boulder OSMP hypothesized that production of a thick thatch cover may be one possible
235 explanation for the plant's success, as it could affect both water and nutrient balances in the soil
236 environment. Our research was designed to explore this and other potential controls and
237 feedbacks on soil N cycling.

238 Our study quantified rates of soil N cycling processes (net N mineralization and net
239 nitrification), as well as a suite of soil properties, aboveground biomass, and leaf tissue C:N
240 ratios at three sites in the Colorado Front Range that typify locations where *A. elatius* has
241 invaded. In general, the net N rates that we observed are consistent with patterns in other
242 ecosystems along the Colorado Front Range: net N mineralization rates exceed atmospheric N
243 deposition of $\sim 4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (e.g., Crawford et al. 2020, Chen et al. 2020, Wetherbee et al.
244 2019, Fisk and Schmidt 1995), highlighting the importance of this process for releasing
245 biologically available N in the soil. Two of our sampling periods provided particularly important
246 insights into variation in soil N cycling between invaded and uninvaded areas and we focus our
247 discussion on them: summer, during peak biomass, and fall, following senescence of *A. elatius*
248 and other grass species (Figures 2 and 3). We found that indeed, there was a trend toward higher
249 rates of both net N mineralization and net nitrification in two of the three sites that we studied:
250 Coyote Canyon, a steep ravine, and NCAR, an open grassland area, in both summer and fall
251 (Figures 2 and 3). We believe that the same pattern was not observed at Shanahan Ridge because

252 plots were located across more variable soil and vegetation environments, including some under
253 the canopy of coniferous trees, which often have lower rates of net N cycling processes
254 (Compton et al. 1998; Aber et al. 1991 and citations therein). In addition, there was also a greater
255 presence of other invasive species in both *A. elatius* invaded and uninvaded plots (e.g., *Bromus*
256 *japonicus*), which may affect soil N cycling.

257 Although soil moisture levels (a first-order control on microbial activity) were slightly
258 lower in summer than during the spring or fall sampling periods (Table 3), microbes were active
259 and released inorganic N in excess of their metabolic demands (i.e., $> 0 \text{ mg N m}^{-2} \text{ day}^{-1}$), with
260 more soil inorganic N production in invaded than uninvaded areas. Measurement of
261 comparatively higher rates of net N cycling processes in summer is consistent with patterns
262 observed by McCulley et al. (2009) in short-, mixed, and tallgrass prairie systems, as well as
263 Owen et al. (2003) in a Taiwanese grassland system. However, it is contrary to Liu et al. (2010)
264 who observed the lowest net N cycling rates during the growing season in temperate grasslands
265 of Inner Mongolia.

266 In contrast, the patterns in net N cycling rates that we observed during the fall sampling
267 period indicate potential consequences for changes to the ecosystem N balance. During fall, net
268 N cycling rates were at least three-fold lower than during the summer but remained positive
269 (indicating release of inorganic N into the soil) in invaded areas. However, in uninvaded areas,
270 net N mineralization rates at Coyote Canyon, and both net N rates at NCAR, were negative,
271 indicating microbial immobilization of N (Figures 2b and 3b). A tendency toward high rates of
272 net N immobilization is consistent with previous studies in tallgrass prairie systems (see
273 McCulley et al. 2009, Risser and Parton 1982 and citations therein). Although not statistically
274 significant, our results suggest that the soil N cycle in uninvaded areas becomes closed when

275 plants senesce in the fall; that is, any inorganic N released by microbes is immobilized in their
276 tissues and remains within the soil system. However, microbes in *A. elatius*-invaded areas may
277 still release inorganic N in excess of their metabolic demand that can be subject to other
278 ecosystem fates or remain in the soil, leaving it enriched for the start of the following growing
279 season.

280 It is important to consider the potential fates of inorganic N released into the soil. While
281 we did not observe a consistent pattern in inorganic N pools across sites, invasion level, or
282 season, they do provide some important insights about variation in soil N dynamics across Front
283 Range grassland systems. Overall, soil inorganic N pools were within the range of those reported
284 for comparable tallgrass prairie systems (McCulley et al. 2009). Interestingly, of the three sites,
285 Shanahan Ridge had the highest inorganic N pools in both summer and fall (Figures 4 and 5),
286 with invaded sites tending to have higher soil inorganic N on average than uninvaded sites. Yet,
287 Shanahan Ridge also tended to have lower net N rates than the other sites, particularly in invaded
288 areas (Figures 2 and 3). In contrast, NCAR, the site with greatest percent cover of *A. elatius* in
289 invaded areas (Table 1) and comparatively higher net N rates, had lower inorganic N pools
290 overall. There, invaded areas had more inorganic N during the summer than uninvaded areas,
291 while the opposite pattern occurred in the fall (Figures 4 and 5).

292 These contrasting examples suggest that the dynamics of both the plant and microbial
293 communities likely differ by site. At Shanahan Ridge, it appears that any inorganic N produced
294 is stored longer in the soil than at the other two sites. At NCAR, net N rates are higher and
295 inorganic N produced may be assimilated by the more densely populated *A. elatius* (e.g., during
296 fall tillering), leading to less inorganic N stored in the soil. In the case of both sites, stored
297 inorganic N could be subject to other ecosystem fates with the addition of snowmelt or rainfall.

298 Past research in California grasslands has highlighted the seasonal pattern of high nitrification
299 rates with the onset of winter rains following dry summers (see Parker and Schimel 2011, Xiang
300 et al. 2011, Fierer and Schimel 2002) and during significant rainfall events in Patagonian
301 grasslands, as observed by Yahdjian et al. (2010). In addition, stored inorganic N may be subject
302 to ecosystem fates that we did not measure, such as leaching or denitrification to the atmosphere.
303 Of the three sites, it appears that there is the greatest potential at Shanahan Ridge for significant
304 episodic transformations of N with the addition of water, or stimulation of N loss from the soil
305 (via denitrification or leaching). More broadly, it is worth noting that there may be important
306 interactions among elevated atmospheric N deposition, plant invasion, and changes to the
307 community of N fixing plants or microbes, which could affect inorganic N pools at these sites
308 (e.g., Wang et al. 2017, Patra et al. 2007).

309 *Possible explanations for trends in soil N cycling rates*

310 An important question that follows from our observations at Coyote Canyon and NCAR
311 is: Why does *A. elatius* seem to accelerate soil N cycling rates? Although our study was not
312 designed to test or demonstrate causality, we did measure a number of factors that could help to
313 explain higher rates in invaded areas. In particular, we were interested in quantifying
314 aboveground biomass production, including thatch, in invaded compared with uninvaded areas
315 (e.g., Stanley et al. 2011). We were also interested in exploring whether *A. elatius* had higher
316 leaf/litter N content than grass species present in uninvaded areas. As demonstrated primarily
317 through studies of N saturation in forested ecosystems (Aber et al. 1998), higher leaf/litter N
318 content can result in accelerated N cycling processes. In addition, more thatch cover can alter
319 soil moisture content by minimizing evaporative losses and holding more water locally (Liang et
320 al. 2017), a particularly important control on tallgrass prairie species (Craine et al. 2010). Thatch

321 cover can also limit rates of primary productivity in native tallgrass species by reducing
322 photosynthetically active radiation to growing shoots and preventing cooling of emergent leaves
323 (Knapp and Seastedt 1986).

324 The hypothesis that *A. elatius* produces more thatch than native prairie communities,
325 which could have feedbacks to increasing rates of net N cycling processes, prompted an
326 aboveground biomass harvest in September 2019. While not statistically significant, we did
327 measure approximately two-fold higher aboveground biomass at Coyote Canyon and NCAR in
328 invaded areas compared with uninvaded areas; at Shanahan Ridge, the difference between
329 invaded and uninvaded areas was less dramatic, but the site exhibited the same pattern as the
330 others, with invaded areas $\sim 200 \text{ g m}^{-2}$ greater in aboveground biomass than uninvaded areas
331 (Table 1). Stanley et al. (2011) also observed higher thatch production by *A. elatius* and other
332 exotic species compared with native plant species in their Pacific Northwest (U.S.) study.
333 Interestingly, the patterns in soil moisture during the fall season did not differ substantially
334 between invaded and uninvaded plots (Table 2). However, our snapshots of soil moisture during
335 soil sampling—particularly right after rain or snowmelt events when we would not observe
336 differences in evaporation or drying time—are insufficient to determine whether or not *A. elatius*
337 changes soil moisture storage due to its thatch cover; this question remains outstanding and
338 requires further investigation.

339 We also considered the C:N ratios of aboveground biomass tissues (leaves + litter), as an
340 indication of whether or not *A. elatius* is N-enriched relative to native prairie grasses. Only at
341 Shanahan Ridge did we find slightly lower C:N ratios in invaded compared with uninvaded
342 areas, but in all other cases, we observed the opposite pattern. Our results are challenging to
343 interpret, due to the timing of our aboveground biomass harvests during fall. On one hand, it is

344 important to consider that by our sampling period in September, *A. elatius* and native grasses had
345 senesced. Thus, it is likely that they had already translocated nutrients to belowground tissues,
346 leaving their aboveground tissues nutrient depleted and fibrous; indeed, previous studies of *A.*
347 *elatius* have noted these qualities once aboveground tissues dry (Sharrow and Syed 2004). On
348 the other hand, previous studies of tallgrass prairie systems report aboveground biomass and
349 litter C:N ratios of ~46, comparable to ours (McCulley et al. 2009). However, these previous
350 studies did not include *A. elatius*-dominated systems. To better understand differences in
351 aboveground biomass C:N ratios, it would be important to collect them during peak biomass.

352 Finally, we also considered soil C:N ratios, as a more stable indicator of long-term effects
353 of *A. elatius* on the soil N environment. Across sites, there was some variability in soil C:N
354 ratios, from ~14 at NCAR to 17 at Coyote Canyon; these values are higher than those reported
355 for Konza Prairie, for example, at 11.1 ± 0.3 (McCulley et al. 2009), and in other areas of the
356 Colorado Front Range, 11.62 ± 0.21 (see Concilio et al. 2015), which may be due to the
357 influence of woody species at our sites. However, averages within invaded and uninvaded areas
358 were virtually the same. This result suggests that *A. elatius* invasion does not affect the
359 stoichiometry of stored soil C and N.

360 Across sites, we did not observe consistent patterns in soil and vegetation characteristics
361 related to trends in net N cycling rates in invaded versus uninvaded areas. Similar complexity has
362 been noted in studies evaluating the effects of another invasive species common to the Colorado
363 Front Range, cheatgrass (*Bromus tectorum*) (Concilio et al. 2015). However, the differences
364 observed in measured variables between invaded and uninvaded areas and among sites (e.g.,
365 Shanahan Ridge had markedly different patterns than Coyote Canyon and NCAR), leads us to
366 some important conclusions and logical next steps. First, the inconsistent directional difference

367 between net N cycling rates in invaded and uninvaded areas across sites suggests that while one
368 might observe similar, high-density *A. elatius* growth across local environments in the foothills
369 of the Colorado Front Range, the effects of that growth on the soil N cycling and ecosystem
370 processes are not the same at every location. By extension, then, the management considerations
371 may differ by site; for example, if one were to conduct a native plant species restoration
372 campaign, the success of that species may differ among areas previously invaded by *A. elatius*.

373 The measurement of net N cycling rates gives a window into the activity of soil microbial
374 communities involved in converting organic N into plant available forms, ammonium and nitrate.
375 The differences in rates that we observed, particularly during the summer and fall sampling
376 periods, suggest that soil microbial community composition and dynamics may differ in *A.*
377 *elatius*-invaded areas compared with native prairie communities. Indeed, several past studies
378 have reported that invasive plant species can alter microbial communities associated with
379 different key N cycling processes, such as N fixation (Xu et al. 2012) and nitrification
380 (Dassonville et al. 2011). An interesting next step would be to evaluate differences in soil
381 microbial community composition and function in invaded and uninvaded areas, and to
382 determine how quickly soil microbial community dynamics change if *A. elatius* is removed and
383 native prairie species are reintroduced.

384 *Limitations of the research*

385 There are a few important limitations of our study that are worth acknowledging, given
386 that they affect how we interpret our data, as well as our ability to extend our results to inform
387 management actions by Boulder OSMP. One consideration is the limited sampling design. We
388 chose to capture patterns of net N cycling rates at three sites typical of those invaded by *A.*
389 *elatius* in the foothills of the Colorado Front Range and to collect samples during three important

390 seasonal periods at each site. Thus, while we did capture variability in response across a range of
391 relevant sites and seasonal states, this came with the tradeoff of reduced sampling (i.e., number
392 of plots) per site per treatment (i.e., invaded versus uninvaded). Rates of net N cycling are
393 notoriously variable spatially, as Hinckley and colleagues (2017a, 2017b) have shown in other
394 parts of the Colorado Front Range. Thus, it is not a surprise that our ability to detect a signal of
395 *A. elatius* was limited; it is perhaps more surprising that given our limited sampling, we did
396 observe some compelling trends.

397 A second important consideration is that we conducted our aboveground biomass harvest
398 (and made measurements of plant tissue C:N ratios) after *A. elatius* and other grasses had
399 senesced. We acknowledge that results may be very different if aboveground biomass harvests
400 are collected during peak biomass in May/June, and also if we had separated stems and leaves
401 from thatch. Similarly, measurement of N content in plant roots may reveal important differences
402 between invaded and uninvaded areas, but we did not make those measurements in our study.
403 Previous research by Boulder OSMP staff noted that *A. elatius* roots can penetrate to 15 cm
404 (below the limit of our 10 cm sampling depth), which may affect microbial activity and soil C
405 and N stocks in ways that we did not capture in this study. Further study of the ways in which *A.*
406 *elatius* roots affect nutrient and water balances compared with uninvaded areas would be useful.

407 Finally, there may be other factors associated with growth of *A. elatius* that affect the
408 persistence of native tallgrass prairie grasses, but we did not measure them, as our primary focus
409 was on soil N cycling. Therefore, it would be inappropriate to conclude from our study that
410 generally, there is a minimal effect of *A. elatius* on the soil environment or growing conditions
411 for native species. Notwithstanding these caveats and limitations, our results provide a
412 foundation for considering how *A. elatius* changes ecosystem N cycling and informing next steps

413 with respect to management efforts by Boulder OSMP—the intended goals of the one-year
414 study.

415 *Recommended next steps for A. elatius management by Boulder OSMP*

416 Our study yielded important information: evidence that in soils covered by 40-80% *A.*
417 *elatius*, rates of net N mineralization and nitrification tended to be higher (with exceptions at one
418 site) than those without *A. elatius* present. While there are interesting studies that could be
419 conducted next to determine whether or not excess inorganic N is assimilated by plants or lost
420 (via denitrification or leaching) to air or water systems—particularly during the fall—more
421 research on the details of N cycling is not the logical next step to inform management actions or
422 goals. Instead, we recommend that Boulder OSMP support trials to determine whether native
423 grass species of interest, such as Big Bluestem (*Andropogon gerardii*) can persist in soils
424 previously colonized by *A. elatius*. Many native grasses like *A. gerardii* are adapted to low-N
425 soil environments (see Averett et al. 2004). Thus, a native species restoration planting study in
426 formerly *A. elatius* invaded areas would help to illuminate whether or not native species could
427 survive. Such an investigation could be coupled to soil biogeochemical measurements that assess
428 how quickly the soil environment transitions to a former, low-N state.

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598 **Table 1.** Location, soil (mean \pm SD), and vegetation (mean \pm SD) characteristics of invaded and uninvaded plots at each site location.
 599 Dominant plant species had >20 % areal cover within plots. Soil type determined from NRCS survey area Version 17, June 5, 2020.

Site	X Coord. (WGS1984)	Y Coord. (WGS1984)	Slope (%)	Soil type	Bulk density (g/cm ³)	pH	Soil C:N	Soil total N (%)	Above Ground Biomass C:N	Above Ground Biomass N (%)	Dominant plant species	<i>A. elatius</i> cover (%)
Coyote Invaded	-105.2804	39.9906	28	Colluvial land, gravelly sandy loam	1.07	6.49 \pm 0.16	16.4 \pm 1.9	0.28 \pm 0.01	47.1 \pm 3.1	0.81 \pm 0.05	<i>Arrhenatherum elatius, Lupinus argentus, Poa aggasizensis</i>	61 \pm 22
Coyote Uninvaded	-105.2804	39.9906	28	Colluvial land, gravelly sandy loam	1.02	6.25 \pm 0.60	17.0 \pm 1.4	0.24 \pm 0.04	45.1 \pm 3.7	0.98 \pm 0.05	<i>Andropogon gerardii, Poa aggasizensis, Bromus japonicus</i>	0
NCAR Invaded	-105.2711	39.9687	15	Colluvial land, gravelly sandy loam	1.02	6.40 \pm 0.15	13.5 \pm 0.6	0.45 \pm 0.04	51.2 \pm 8.3	0.78 \pm 0.06	<i>Arrhenatherum elatius, Poa aggasizensis</i>	66 \pm 22
NCAR Uninvaded	-105.2706	39.9691	15	Colluvial land, gravelly sandy loam	0.89	6.69 \pm 0.17	13.5 \pm 1.1	0.40 \pm 0.01	37.1 \pm 5.8	0.95 \pm 0.11	<i>Bromus japonicus, Poa compressa, Poa aggasizensis,</i>	0
Shanahan Invaded	-105.2681	39.9643	1	Nederland very cobbly sandy loam	1.12	6.17 \pm 0.53	15.6 \pm 3.7	0.42 \pm 0.04	49.0 \pm 3.0	0.93 \pm 0.06	<i>Arrhenatherum elatius, Andropogon gerardii</i>	52 \pm 23
Shanahan Uninvaded	-105.2677	39.9644	1	Nederland very cobbly sandy loam	0.83	6.17 \pm 0.21	15.4 \pm 2.7	0.34 \pm 0.04	58.9 \pm 8.9	0.77 \pm 0.06	<i>Bromus japonicus, Pascopyrum smithii, Poa aggasizensis, Virgulus falcatus, Poa compressa</i>	0

600

601

602 **Table 2.** Differences in average net N cycling and N pools between uninvaded and invaded plots across seasons and sites. The
 603 asterisk indicates statistical significance between uninvaded and invaded plots.

Site	Summer 2020				Fall 2020			
	Net mineralization rate difference (uninvaded- invaded)	Net nitrification rate difference (uninvaded- invaded)	Ammonium pool difference (uninvaded- invaded)	Nitrate pool difference (uninvaded- invaded)	Net mineralization rate difference (uninvaded- invaded)	Net nitrification rate difference (uninvaded- invaded)	Ammonium pool difference (uninvaded- invaded)	Nitrate pool difference (uninvaded- invaded)
Coyote	-58.2	-51.8	123.1	64.6	-6.1	-0.9	160.9	103.5
NCAR	-26.4 *	-23.3	-18.3	-29.5	-21.8	-16.7	181.9	72.5
Shanahan	24.2	-0.9	-507.5	-91.2	1.0	-6.0	-331.8	-365.8

* $P < 0.05$

604
605

606 **Table 3.** Average soil moisture content (mean \pm SD).

Site/Invasion	Spring (% water)	Summer (% water)	Fall (% water)
Coyote			
Invaded	20.12 \pm 2.03	12.56 \pm 4.94	14.81 \pm 3.56
Uninvaded	14.16 \pm 1.92	10.25 \pm 2.50	13.46 \pm 2.47
NCAR			
Invaded	23.08 \pm 1.41	18.39 \pm 1.75	23.84 \pm 4.69
Uninvaded	23.35 \pm 1.8	16.82 \pm 2.89	23.05 \pm 4.92
Shanahan			
Invaded	21.50 \pm 3.85	15.72 \pm 6.59	17.25 \pm 4.45
Uninvaded	22.52 \pm 0.57	18.71 \pm 1.98	18.57 \pm 33.24

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608

609



Coyote



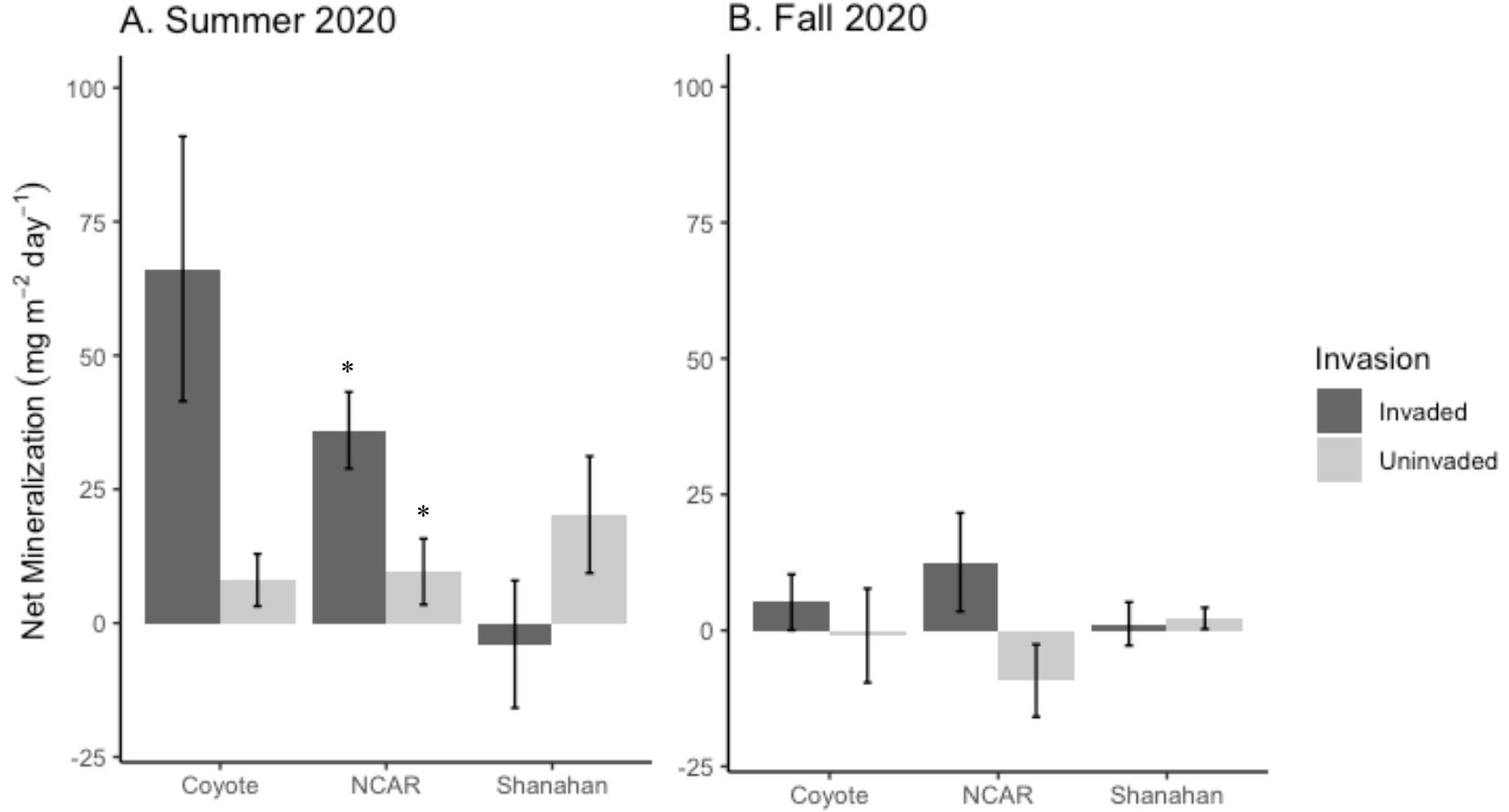
NCAR



Shanahan

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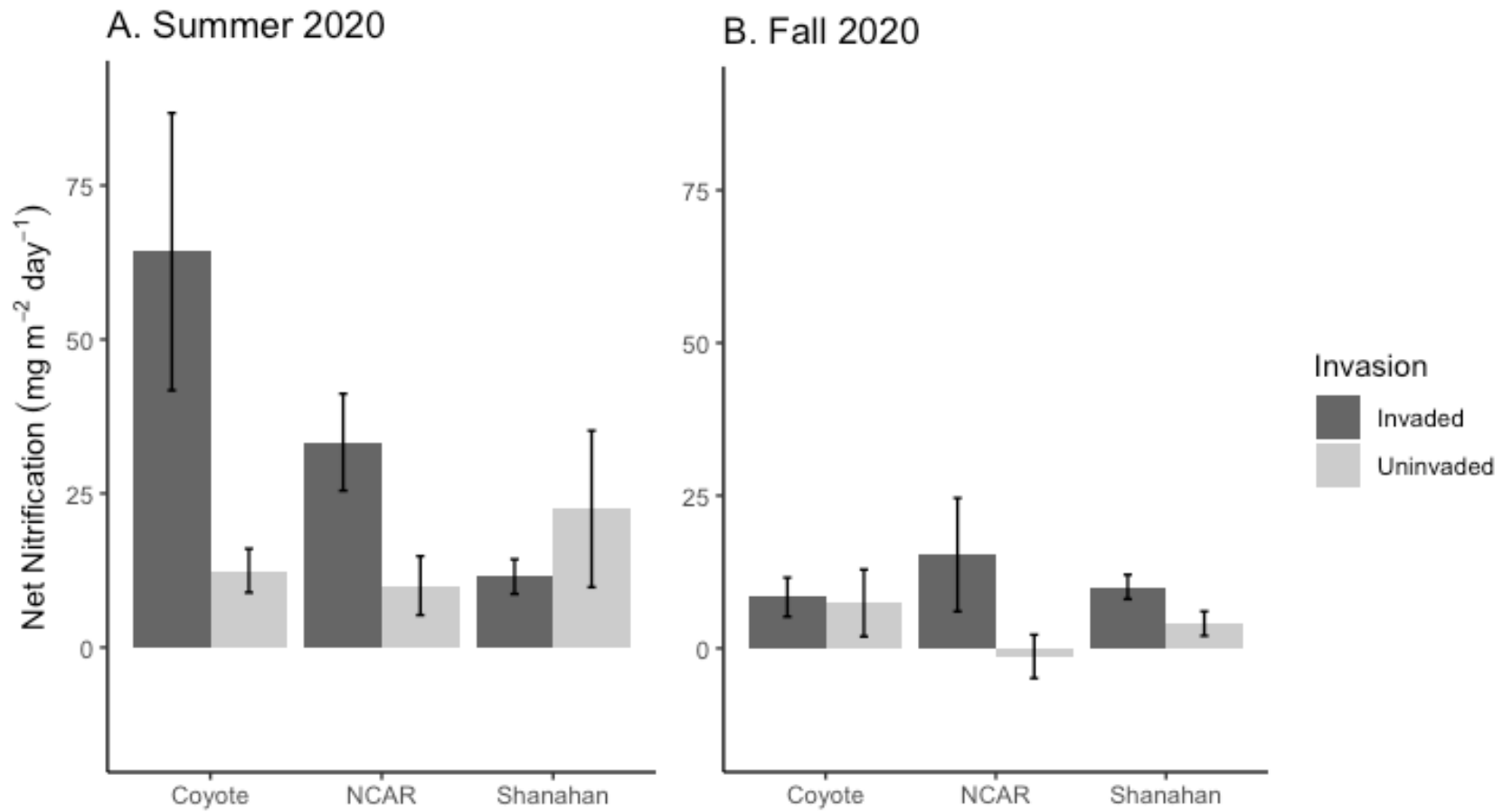
611 **Figure 1.** Site map with images from each location.



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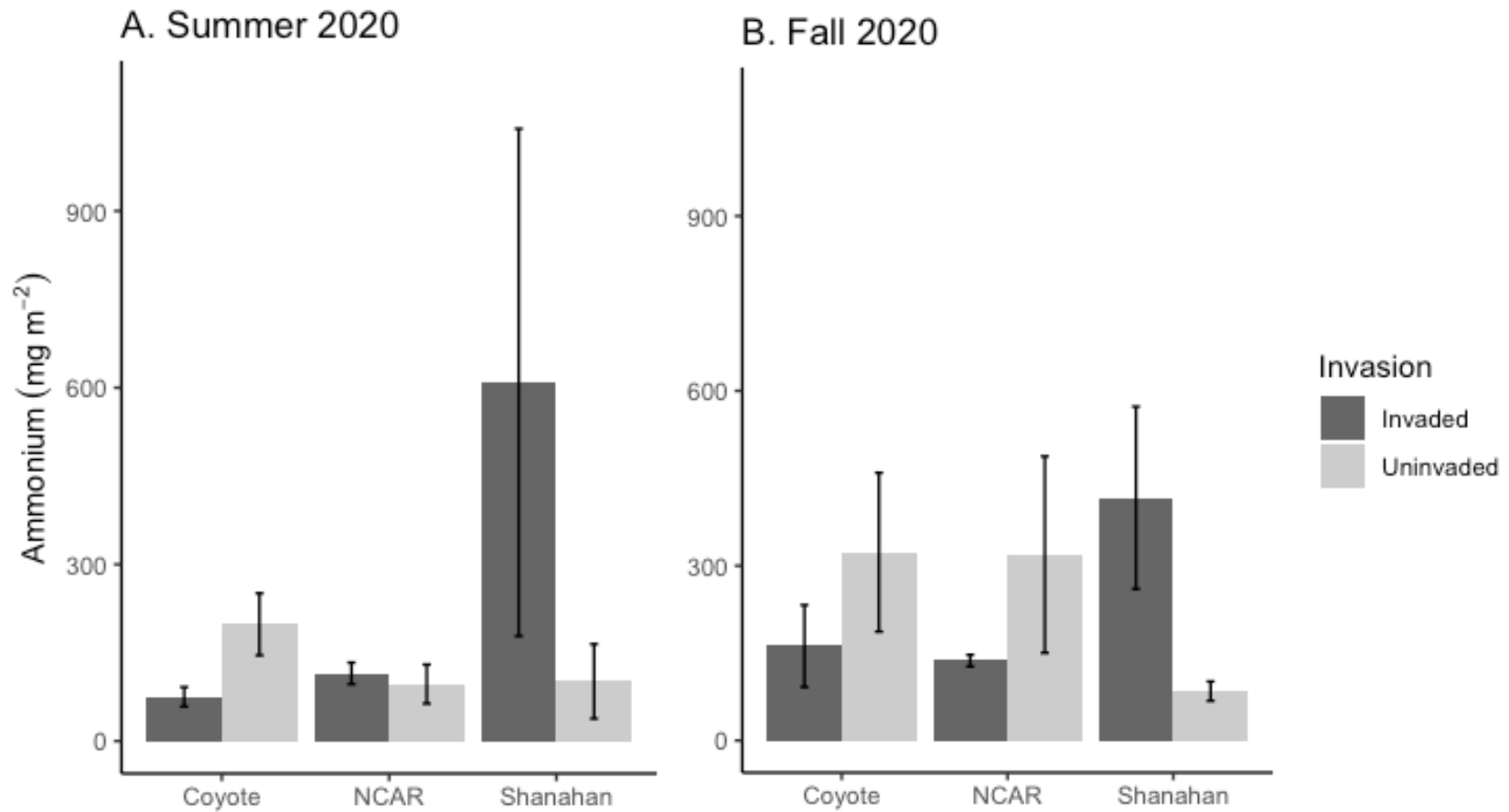
614 **Figure 2.** Mean (\pm SD) net N mineralization rates in invaded and uninvaded plots for Summer 2020 (A) and Fall 2020 (B). The
 615 asterisk indicates statistical significance between invaded and uninvaded plots at each site.



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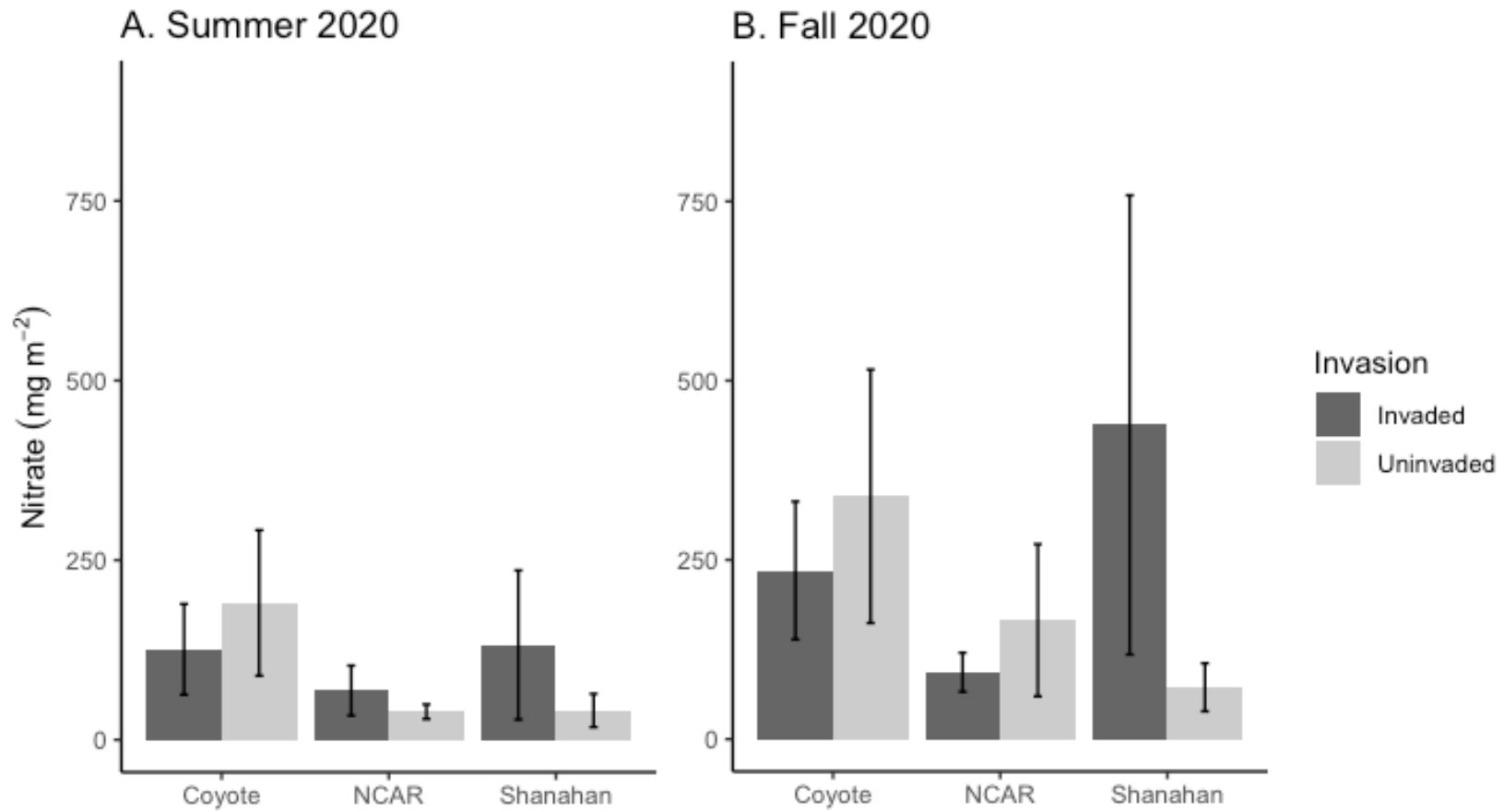
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618 **Figure 3.** Mean (\pm SD) net N nitrification rates in invaded and uninvaded plots for Summer 2020 (A) and Fall 2020 (B). No sites had
 619 a significant statistical difference between invaded and uninvaded plots ($p > 0.05$).



605

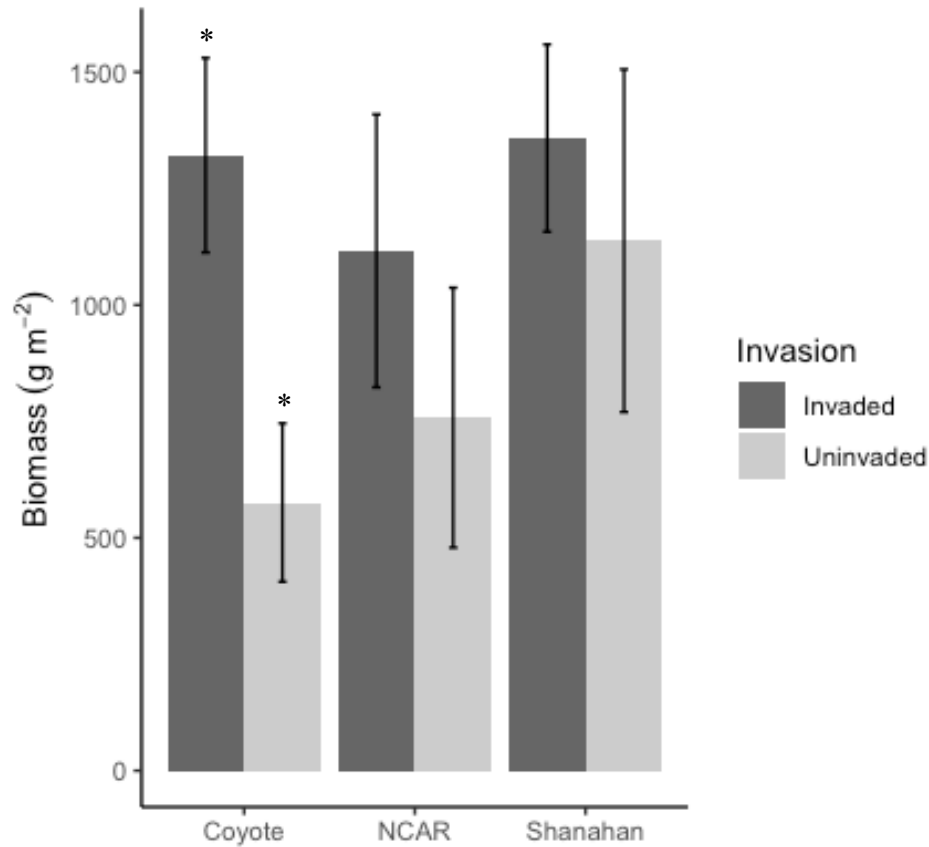
606 **Figure 4.** Mean (\pm SD) ammonium pools in invaded and uninvaded plots for Summer 2020 (A) and Fall 2020 (B). No sites had a
 607 significant statistical difference between invaded and uninvaded plots ($p > 0.05$).



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609

610 **Figure 5.** Mean (\pm SD) nitrate pools in invaded and uninvaded plots for Summer 2020 (A) and Fall 2020 (B). No sites had a
611 significant statistical difference between invaded and uninvaded plots ($p > 0.05$).



612

613 **Figure 6.** Mean (\pm SD) above ground biomass in invaded and uninvaded plots at each site. The
614 asterisk indicates statistical significance between invaded and uninvaded plots at each site ($p <$
615 0.05).