1 Title page

2

3 **Soil enzymes associated with carbon and nitrogen cycling in invaded and native secondary forests of**
4 **northwestern Argentina**

5

6 Roxana Aragón[1*], Jordi Sardans[2,3] and Josep Peñuelas[2,3].

7

8 [1] Instituto de Ecología Regional, Universidad Nacional de Tucumán. CC 34 (4107) Tucumán, Argentina
9 [2]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain.
10 [3]CREAF, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain.

11

12 *Corresponding author: roxaragon@gmail.com. Instituto de Ecología Regional (IER), Universidad Nacional
13 de Tucumán. CC 34 (4107) Tucumán, Argentina. Tel/fax: +54381-4255174

14

15 Email addresses of co-authors:
16 Jordi Sardans: j.sardans@creaf.uab.cat; Josep Peñuelas: josep.penuelas@uab.cat

17

18

19 **Abstract**
20

21 *Background and Aims* Alien success has frequently been associated with changes in the concentrations of soil
22 nutrients. We aim to investigate the effects of plant invasion on soil nutrients, potential enzyme activity and
23 litter elemental composition and stoichiometry.
24 *Methods* We compared stands of secondary forest invaded by *Ligustrum lucidum* and those dominated by
25 natives, and performed litter and soil chemical analyses on 3 native and 2 exotic tree species.
26 *Results* Soils of invaded sites had 20% and 30% increase in β-glucosidase and alkaline phosphatase activity,
27 higher Olsen-phosphorus (P) and potassium (K) concentrations and lower nitrogen (N) concentration and N:P,
28 N:K and ammonium:Olsen-P ratios. Invaded and non-invaded sites differed in their overall nutrient
29 composition and enzyme activity. Natives and exotics differed in nine of the 16 litter elemental composition
30 and stoichiometry variables analyzed.
31 *Conclusions* The low N:P ratio in litter, the decrease in soil N in invaded stands and the low N concentration
32 of exotics suggest that N is the limiting nutrient and that exotic success is related to higher N uptake and use
33 efficiency. The higher investment in the acquisition of soil resources, higher nutrient uptake and use
34 efficiency of limiting nutrients contribute to the success of exotics in this subtropical forest.
35
36
37 Keywords: soil enzyme, nitrogen, phosphorus, litter, stoichiometry, *Ligustrum lucidum*
38

39 **Introduction**

40 Litter decomposition in terrestrial ecosystems is largely the result of the activity of soil enzymes from
41 communities of bacteria and fungi. This activity is in turn conditioned by physical factors (e.g. temperature,
42 soil humidity and soil pH) and litter characteristics (Sinsabaugh et al. 1993; Kourtev et al. 2002, Tharayil et
43 al. 2013). Vegetational cover modifies both environmental and litter characteristics and consequently the
44 abundance, diversity and activity of microbial communities (van der Putten et al. 2007). Importantly,
45 invasions by exotic species represent a rapid change in community composition and are thus likely to affect
46 the control of litter decomposition and, in turn, the concentrations of soil nutrients and stoichiometric
47 relationships (Ehrenfeld et al. 2001; Ehrenfeld 2003; Allison and Vitousek 2004; Joanisse et al. 2007; Flory
48 and Clay 2010).

49 β-glucosidases, proteases, ureases and phosphatases are the most important soil enzymes involved in the
50 carbon (C), nitrogen (N) and phosphorus (P) cycles in the soil (Sardans and Peñuelas 2005; Sardans et al.
51 2008). β-glucosidases break down labile cellulose and related carbohydrates with 1-4 glucosidic bonds,
52 degrading plant cell walls and thus contributing to the firsts phases of plant cell tissues decomposition, which
53 then facilitate the activities of other enzymes such as proteases and phosphatases (Debosz et al. 1999; Sardans
54 et al. 2008; Stege et al. 2010). Proteases are involved in the first phase of N mineralization by hydrolyzing the
55 peptide bonds of amino acids. Ureases regulate the release of $N\text{-}NH_4$ by urea hydrolysis, which is essential in
56 the chain of hydrolysis of amino compounds. Phosphatases regulate the hydrolysis of O-P bonds, releasing
57 orthophosphate from organic matter (Sardans and Peñuelas 2005).

58 Water and resource limitation partly constrain the production and activities of soil enzymes (Criquet
59 et al. 2004; Allison and Vitousek 2005; Sardans and Peñuelas 2010). Plant invasions generate changes in a
60 forest's capacity to take up and use soil resources (water and nutrients) by both introducing a new species and
61 by modifying the capacities of native species, hence invasions can modify soil nutrient and water contents,
62 that in turn may affect the activities of soil enzymes (Kolb et al. 2002; Ehrenfeld 2003; Allison and Vitousek
63 2004; Joanisse et al. 2007). Several studies have described the effects of plant invasion on the concentrations
64 of soil nutrients (Evans et al. 2001; Allison and Vitousek 2004) but little is known about its effects on soil
65 stoichiometry and the mechanisms underlying these changes (Sardans and Peñuelas 2012). The objective of
66 the present study was to discern the impacts of plant invasion on the activities of soil enzymes and on soil and
67 litter nutrient concentrations and stoichiometry by studying forest stands in sites heavily invaded by
68 *Ligustrum lucidum* (Oleaceae) and in sites dominated by native species in a montane forest of northwestern
69 Argentina.

70 *L. lucidum* is an evergreen, shade-tolerant tree with high resprouting capacity, survival and growth
71 rates (Easdale et al. 2007). Invasion by *Ligustrum* modifies soil moisture, light availability, litter depth and
72 plant diversity (Lichstein et al. 2004; Aragón et al. 2014; Ayup et al. 2014). The litter of this invasive species
73 has a significantly higher decomposition rate than those of three of the most common native species in the
74 area (Aragón et al. 2014). Here we analyzed, for the first time to the best of our knowledge, the effects of
75 plant invasion on the elemental composition and stoichiometry of both soil and litter and on the activities of
76 soil enzymes. Given *Ligustrum* high growth rate, we hypothesize a potentially high demand of resources. In

77 addition to *Ligustrum* demands, the environmental conditions (i.e., lower soil moisture and light availability)
78 present in invaded stands, could lead to increases in soil-enzyme concentration in order to compensate for the
79 unfavorable conditions. All these in turn, could result in increases in soil and litter nutrient concentrations
80 and in changes in soil and litter stoichiometries.

81

82 **Methods**

83 **Study Site**

84 The study was conducted in the lower montane forest of Sierra de San Javier, Tucumán, Argentina (26º 70' S,
85 65º 35' W) at approximately 800 m a.s.l. The area represents the southern-most limit of the subtropical
86 Andean montane forest (also known as *Yungas*), which extends from Bolivia to the province of Catamarca in
87 Argentina (Cabrera and Willink 1980; Grau and Brown 1995). Average annual precipitation ranges from 1300
88 to 1500 mm distributed in a monsoonal regime with dry winters and wet summers (Bianchi and Yañez, 1992).
89 The mean annual temperature is 18 ºC, with frosts occurring from June to August. Most of the Sierra de San
90 Javier piedmont was cleared for crop production and grazing during the early twentieth century (Grau and
91 Brown 1995; Brown et al. 2001), but many cleared areas were abandoned in the last two decades and
92 currently have forests at different stages of regeneration (Grau and Aide 2007; Grau et al. 2008). Many of
93 these secondary forest stands are colonized by several exotic species (Grau and Aragón 2000), but *L. lucidum*
94 is by far the most abundant exotic species and the only one that forms monodominant forest stands in this
95 area.

96

97 **Studied species**

98 We included five tree species in this study: two exotics (*L. lucidum* and *Morus* sp.) and three natives
99 (*Cinnamomum porphyrium, Cupania vernalis and Myrsine laetevirens*). *L. lucidum* is an Asian tree that
100 colonizes areas of varying land-use histories and ages. It is more abundant in secondary forest stands but also
101 grows in openings in old-growth forests (Aragón and Morales 2003). *L. lucidum* is evergreen, shade tolerant
102 and has a high growth rate (Easdale et al. 2007). Importantly, its distribution is expected to expand in the near
103 future (Grau et al. 2008). *Morus* sp. is also of Asian origin, but unlike *Ligustrum*, it is deciduous and shade
104 intolerant (Grau et al. 1997; Easdale et al. 2007). *Morus* sp. is a fast-growing species as well and is most
105 abundant on the edges of young secondary forest stands that were reclaimed from citrus orchards.

106 The native species in this study are all late successional and bird dispersed, with relatively high growth
107 rates compared to the other native species in this area (these species are among the 10 fastest-growing species
108 among a set of 29 species studied by Easdale et al. (2007)). They are also among the most abundant in the
109 canopy or subcanopy strata (Grau et al. 1997; Easdale et al. 2007). *C. porphyrium* is a semi-deciduous, tall,
110 shade-tolerant tree abundant in the canopies of secondary and old-growth forests (Grau et al. 1997; Easdale et
111 al. 2007). *C. vernalis* has similar life-history characteristics, but it integrates into the subcanopy stratum. Its
112 saplings account for approximately 70% of the saplings in native and invaded forest understories (Grau et al.
113 1997; Lichstein et al. 2004). *M. laetevirens* is an evergreen tree, of intermediate height (it sometimes
114 integrates into the canopy) and has the highest growth rate among the three native species studied (Easdale et

115 al. 2007). Unlike the other species, *Myrsine* bears fruit during the winter, partially coinciding with *L. lucidum*
116 fructification. The morphological and demographic characteristics of *Myrsine* are also more similar to those
117 of *Ligustrum* (e.g. maximum growth rate, growth in well-lit conditions and density in secondary forests)
118 (Easdale et al. 2007).
119

120 **Experimental design and sampling**

121 To evaluate the activities of soil enzymes in native and invaded forests, we used a paired design with five
122 invaded-native stand pairs .We considered as invaded those stands which had *L. lucidum* as the dominant
123 species in the canopy and occurring at densities higher than 500 ind./ha. Native stands were dominated by *C.*
124 *porphyrium*, *Blepharocalix salicifolius* and *C. vernalis* among others, and even though some individuals of *L.*
125 *lucidum* were present, especially as saplings, they could be considered rare. Even though *Morus* sp is
126 abundant in many secondary patches, it does not form mono-dominant stands. For this reason, we only
127 considered two forest types: stands invaded by *Ligustrum,* and stands dominated by native species.
128 Importantly, native and exotic species co-occur in the different stands but at very dis-similar abundance. For
129 more details about species composition and stands characteristics see Grau et al. 1997; Aragón and Morales
130 2003 and Easdale et al. 2007. Within each pair, the stands were similar in age (between 30 and 50 years of
131 succession), altitude (between 550 and 700 m), slope and soil type (typically hapludoll with loam sandy
132 texture with 50-30-20 % of loam, sand and clay respectively) and were larger than 2 ha. Pairs were selected
133 based on the greatest similarity in age and the smallest geographic separation (from 200 and 500 m between
134 the members of each pair). For more details about the location of the stands in the field see Aragón et al.
135 (2014). We established a 3 x 3 m plot in each stand, avoiding edges and gaps in the canopy.

136 Three soil cores (diameter 6 cm) from the top 10 cm of the soil profile were collected from each plot of
137 the five invaded/native pairs in April 2012. Each soil sample was kept at approximately 5 ºC until analyzed.
138 In the laboratory, we first sieved the soil through a 2-mm mesh and then analyzed the two fractions for soil-
139 enzyme activity and concentrations of C and N and the main nutrients Ca, Fe, Mg, Mn, and Na. For the soil
140 analyses (i.e., enzymes and nutrients) five pairs of stands (invaded and non-invaded forests in each pair) in 5
141 different sites were considered to account for the potential site to site variability. Each pair was taken as a
142 block (5 repetitions), and forest types (two levels) as treatment (fixed factor).

143 The litterfall of 3-8 individuals of each of the five studied species was collected in invaded and native sites
144 between May and September 2011 into plastic bags suspended underneath each plant. Leaves were air-dried
145 for 3-5 days and stored in open paper bags until further analysis.
146

147 **Chemical analyses**

148 **Activities of soil enzymes**

149 To determine β-glucosidase activity, we incubated 5 g of soil for 3 h at 37 ºC with acetate buffer (2 M, pH
150 6.2 diluted in 1000 ml of distilled water) and with salicin (β-glucosido-saligenin) as a substrate (Tabatabai
151 1994). The solutions were filtered (Millipore 0.45-µm HA nitrocellulose filter) and the saligenin released
152 from the substrate was determined colorimetrically after coloring with 2,6-dibromomchinon-4-chloroimide in

153 a borate buffer (0.2 M, pH 10). At pHs above 9, saligenin forms a blue indophenol dye with 2,6-
154 dibromchinon-4-chloroimide, which was then measured at 578 nm with a Helios α spectrophotometer
155 (Thermo Scientific, Waltham, MA, USA) against the reagent blank. We calculated the saligenin content by
156 referring to a calibration curve obtained with standards containing 0, 10, 20, 50 and 100 µg of saligenin per
157 ml. *β*-glucosidase activity was expressed as µg of saligenin released per gram of soil per hour. For the
158 analyses of all enzymes we first dried the soil samples by freezing in order to prevent protein damage by heat.

159 To determine protease activity, we used the method of Ladd et al. (1976) using casein as substrate.
160 Briefly, 5 mL of substrate solution (casein 2%, w/w) was added to 1 g of soil sample. We added 5 mL of Tris
161 (Tris-hydroxymethyl-aminomethane) buffer (0.05 M, pH 8.1) and then incubated for 2 h at 50 ºC. After
162 incubation, the remaining substrate was precipitated with trichloroacetic acid. Thereafter, samples and
163 controls were filtered immediately. For photometric analysis, 5 mL of the filtrate was added to 7.5 mL of
164 alkali reagent in a test tube, mixed well, 5 mL of Folin-Ciocalteu's phenol reagent were added, and mixed
165 again. Alkali reagent is a mix of three solutions: a) 50 g of sodium carbonate, 60 mL of 0.1 M NaOH in 600
166 mL of distilled water (1000 mL); b) 5g of copper sulfate pentahydrate in 1000 mL of distilled water (20 mL);
167 and c) 10 g of sodium potassium tartate in 1000 mL of distilled water (20 mL). Before colorimetric
168 measurement, the samples, controls and standards were filtered (Millipore 0.45-µm HA nitrocellulose filter)
169 to prevent interference from the precipitates formed by the casein reaction products. The solutions were then
170 allowed to stand at room temperature for exactly 90 min for color development. We measured the extinction
171 at 700 nm with the spectrophotometer against the reagent blank and calculated the tyrosine content by
172 referring to a calibration curve obtained with standards containing 0, 100, 250, 1000 and 1500 µg of tyrosine
173 per ml. Protease activity was expressed as µg tyrosine per gram of soil per hour.

174 We used the Kandeler and Gerber (1988) method for determining urease activity. An aqueous (controls) or
175 a buffered urea solution (samples) was added to 5 g of soil sample and incubated for 2 h at 37 °C. Released
176 ammonium (NH_4^+) was extracted with 2 mol L^{-1} KCl and quantified by a modified Berthelot reaction
177 (Schinner et al. 1996). The solutions were shaken for 30 min and filtered (Millipore 0.45-µm HA
178 nitrocellulose filter) to prevent interference from possible precipitates. The determination was based on the
179 reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate, which forms a green
180 complex under alkaline pH conditions. The extinction was measured at 690 nm against the reagent blank.
181 Sodium nitroprusside was used as a catalyst to increase the sensitivity of the method approximately 10-fold.
182 We calculated the NH_4^+ content by referring to a calibration curve obtained with standards containing 0, 1,
183 1.5, 2, and 2.5 mg NH_4^+ L^{-1}. Urease activity was expressed as µg NH_4^+ released per gram of soil per hour.

184 Phosphatase activity was determined by adding 4 mL of THAM solution (Tris-hydroxymethyl-
185 aminomethane with citric, maleic and boric acids), buffer (tris hydroxymethil aminomethane, maleic acid,
186 citric acid monohydrate and boric acid in 500 mL of 1 M NaOH, at pH 6.5 for acid phosphatase assays or pH
187 11 for alkaline phosphatase assays) and 1 mL of p-nitrophenyl phosphate solution (as a substrate) prepared in
188 the same buffer to 1 g of soil in a flask. We then swirled the flask for a few seconds to mix the contents. The
189 stoppered flask was incubated at 37 ºC for 1 h, and then1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were

190 added. The flask was again swirled for a few seconds to stop the reaction. The solution was filtered as above
191 to prevent the appearance of possible precipitates. The fading of the intensity of the yellow color in the
192 calibration standards, samples and controls was measured at 398 nm against the reagent blank. We calculated
193 the p-nitrophenol content by referring to a calibration curve obtained with standards containing 0, 10, 20, 30,
194 40 and 50 ppm of p-nitrophenol. Phosphatase activity was expressed as μg p-nitrophenol per gram of soil per
195 hour.
196

197 **Chemical analyses of litter and soil**

198 For chemical analyses of foliar tissue, leaves were dried in an oven at 60 ºC to a constant weight and then
199 ground in a CYCLOTEC 1093 (Foss Tecator, Hoganas, Sweden) and stored in desiccators until analysis. C
200 and N contents were determined from 0.7 mg of pulverized dried sample by combustion coupled to gas
201 chromatography in an Elemental Analyzer CHNS Eurovector 3011 Thermo Electron Gas Chromatograph
202 model NA 2100 (C.E. Instruments-Thermo Electron, Milan, Italy). For the other nutrients (Ca, Fe, Mg, Mn, S,
203 P, K and Na), 0.25 g of pulverized dried sample was diluted with the acid mixture HNO_3 (60%) and H_2O_2
204 (30% w/w) and digested in a MARSXpress microwave system (CEM, Matthews, NC, USA). The digested
205 solutions were brought to a final volume of 50 mL with ultra pure water and at 1% HNO_3. Blank solutions (5
206 mL of HNO_3 with 2 mL H_2O_2 without any sample biomass) were regularly analyzed. After digestion, the
207 concentrations of Ca, Fe, Mg, Mn, S, P, K and Na were analyzed with an Optima 4300DV ICP-OES (Optical
208 Emission Spectrometer for Inductively Coupled Plasma, Perkin-Elmer, Waltham, MA, USA). To assess the
209 accuracy of the biomass digestion and analytical procedures, we used certified biomass NIST 1573a (tomato
210 leaf) standards. To analyze the soil samples, we followed the same protocols used for the foliar tissues, but we
211 filtered the samples with a 0.45 μm microfilter.

212 In addition to C, N and the other nutrients, we also determined immediately available P (Olsen P) and
213 ammonium. The P available to plants in the soil was determined by Olsen's method (Olsen et al. 1954). This
214 method measures the relative availability of orthophosphate (PO_4-P) extracted in 0.5 M $NaHCO_3$ adjusted to
215 pH 8.5. Phosphorus content in 1 g of soil was determined spectrophotometrically at 882 nm in an acidic
216 medium of 0.24 M H_2SO_4 by reacting with ammonium molybdate using ascorbic acid as a reductant in the
217 presence of antimony potassium tartrate. Phosphorus concentration was determined using a calibration curve
218 built with seven solutions containing 0.0, 0.25, 0.50, 0.75, 1, 2 and 3 mg L^{-1} PO_4-P.

219 To determine the ammonium concentration in the soil, we used a procedure similar to that described for
220 urea. Released ammonium (NH_4^+) was extracted with a 2 mol L^{-1} KCl solution added to 1 g of dried soil. The
221 determination was based on the reaction of sodium salicylate with NH_3 in the presence of sodium
222 dichloroisocyanurate and using sodium nitroprusside as a catalyst. The measurement was performed at 690
223 nm using a calibration curve similar to that described for urea.

224 **Statistical Analyses**

225 We analyzed soil-enzyme activities, ammonium and Olsen-P concentrations and ammonium:Olsen-P
226 ratios in the soil with two-way ANOVAs, with forest types as treatments (invaded and native) and the five

227 pairs of sites as blocks (repetitions). In the case of leaf litter, we first considered two sources of variation:
228 forest types (litter collected from individuals occurring in invaded or in native stands: two levels) and species
229 (five levels). Secondly, we explored the differences only among species, and lastly we grouped species in
230 native (three species) and exotics (two species) (i.e., two levels).

231 We performed principal component analyses (PCA) using correlation matrices with 20 variables for the
232 soil (five enzymes, 12 nutrients including ammonium and Olsen P and three ratios) and 13 variables for the
233 litter (10 nutrients and three ratios). The differences among treatments (invaded and native for the soil and
234 five species for the litter) in the variable distribution in the multidimensional space defined by the PCA were
235 tested using an ANOVA for the scores of sites (n=10) or individuals (10 of each species). In addition, to
236 specifically evaluate if the invasion of *L. lucidum* changed over all studied soil variables, nutrient
237 compositions and potential enzyme activities in the soil and nutrient composition in the litter, we used a
238 multi-response permutation procedure (MRPP) based on Euclidean distance (Biondini et al. 1985). MRPP is a
239 non-parametric procedure for testing the null hypothesis of differences between groups or entities. It provides
240 a statistic, δ, that is the weighted mean of within-group distances and is associated with a *p*-value that
241 indicates the likelihood of δ being equal to or smaller than that observed by chance (McCune and Mefford
242 1999). For the soils, we used a block-MRPP considering the five pairs of invaded/native sites, and for the
243 litter, we grouped data from all species and considered forest type as the only source of variation. All the
244 multivariate analyses were performed with the PC-ORD 5 program (McCune and Mefford 1999). In addition,
245 for the litter, we performed univariate ANOVAs with 16 variables (10 nutrients and six ratios) and with
246 species status (native or exotic) as the classification factor. We also made multiple comparisons among
247 species (five levels).

248

249 **Results**

250 **Enzymes and nutrients in the soil**

251 Invaded sites had an approximately 20% increase in β-glucosidase activity and a 30% increase in alkaline
252 phosphatase activity. The soil of invaded sites had a lower N concentration (both as NH_4^+ and total N), higher
253 Olsen-P concentration (40%) and lower ammonium:Olsen-P ratio (67%) (Table 1). Invaded stands also had a
254 higher K concentration (13%) and lower N:P and N:K ratios (25% and 39%, respectively) (Table 2). The first
255 two axes of the PCA ordination that considered 20 variables explained 66% of the total variance (Figure 1).
256 The first axis (explaining 42.8% of the total variance) was related to the variability among blocks, and the
257 second axis (explaining 23.4% of the total variance) separated invaded from native stands (ANOVA with
258 PCA scores: Axis I: F for blocks = 7.1, $p = 0.04$; Axis II: F for forest type = 17.27, $p = 0.01$). Invaded sites
259 were associated with higher enzyme activities and Olsen-P and K concentrations, while the
260 ammonium:Olsen-P ratio and Mg and Fe concentrations were higher in the native sites (Figure 1). This
261 pattern was reinforced by the MRPP results. Overall, the distances within invaded and native stands were
262 smaller than expected by chance (chance-corrected within group agreement = 0.19, $p = 0.03$), so we can

263 conclude that the invaded and native sites significantly differed in their nutrient compositions and enzyme
264 activities.

265 **Chemical analyses of leaf litter**

266 The litter of the different species had similar elemental compositions in the highly invaded stands and the
267 native stands (Table A1, appendix). This pattern was evident from both the univariate and the MRPP analyses
268 (chance-corrected within group agreement = 0.01, $p = 0.15$). In contrast, the litter of the different species had
269 several differences in elemental composition and stoichiometry. The native and exotic species as groups
270 differed in nine of the 16 studied variables (Table 3). Exotics had higher concentrations of Ca and K and
271 lower concentrations of Mn, P, C and N. Differences among species were also evident through the PCA
272 ordination, especially between the natives *Cinnamomum* and *Cupania* and the other studied species, whereas
273 the two exotics and the native *Myrsine* all shared some characteristics (Figure 2a). The first three axes of the
274 PCA ordination explained 63% of the total variability. Two groups could be distinguished along the first axis
275 (29.7% of the variability) (Figure. 2a, letters on the top): exotics and *Myrsine* on the positive side and
276 *Cinnamomum* and *Cupania* on the negative side. Exotics and *Myrsine* had higher concentrations of Ca and K
277 and lower N:K and P:K ratios compared to the remaining two species (Figure 2b). The second axis (20.4% of
278 the variability) separated three groups: *Cupania* with litter concentrations of Mg, Fe, S, Na, P and Mn higher
279 than those for *Cinnamomum* and exotics and *Myrsine* with intermediate characteristics (Fig. 2a letters on the
280 right). The third axis (13.6% of the variability) separated, although to a minor degree, exotics (*Ligustrum* and
281 *Morus*) from *Myrsine* (Figure 3a letters on the right), and overall, the invasive from the native species as
282 groups (Fig 3 a, right arrows) Exotics had lower N concentrations and higher Ca concentrations (Figure 3b)
283 (Table 3).
284

285 **Discussion**

286 **Effects of invasion on soil and litter elemental composition and stoichiometry**

287 The soils of invaded stands had lower N concentrations (both as NH_4^+ and total N), higher extractable K
288 concentrations, higher plant-available P concentrations and a general trend toward higher soil-enzyme
289 activities. All these data suggest higher rates of nutrient cycling, mainly of N, in the invaded stands. These
290 results are in agreement with several studies reporting significant impacts of alien plants on the availability of
291 soil nutrients, decomposition of organic matter, nutrient cycling and soil stoichiometry (Sardans and Peñuelas
292 2012, Tharayil et al. 2013). In a recent review of the effect of invasive plants on N and P availability, C:N:P
293 ratios of soils and rates of soil decomposition, mineralization and nutrient cycling from 65 studies conducted
294 in environments with unclear limitations of nutrients (except some conducted mainly in arid and semiarid
295 areas of the USA), 48 studies reported increases in the availability of soil nutrients, 14 reported decreases and
296 three were inconclusive (Sardans and Peñuelas 2012). Most of the 14 studies reporting decreases in soil
297 nutrients were studies with *Bromus tectorum*, an invasive grass of semiarid areas of the USA (but see Castro-
298 Díez et al. 2013), indicating that most invasions in nutrient-rich ecosystems tend to increase the availability of

299 soil nutrients and hence to increase nutrient cycling. In forests invaded by *Ligustrum*, however, we found a
300 decrease in nutrients, particularly N. Importantly, less is known of the effect of plant invasions on nutrient
301 imbalances. By investigating litter and soil stoichiometry and soil-enzyme activity in Argentine subtropical
302 forests, we have shown that the success of invasive plants is associated with an overall change in soil nutrient
303 composition and function, mainly by a decrease in the most limiting nutrient, here N, increasing the
304 imbalances with other nutrients such as P, which tends to increase its availability in the soil (i.e. decreasing
305 N:P ratio). This result sheds light on the role of N:P ratios in plant invasions, which has remained
306 inconclusive (Sardans and Peñuelas 2012).

307 Even though we cannot effectively discriminate plant available N with our data, there are several
308 indications that N appears to be the most limiting element in this area. The global litter N:P ratio is 46:1
309 (±3:1) (McGroddy et al. 2004) but was 7.4:1 (± 1:1) in our study. Moreover, the lower soil water content in
310 invaded stands was associated with lower total N concentrations, suggesting that invasive success is related to
311 higher N uptake. In contrast, P in these soils did not appear to be limiting for plant growth. Invaded stands had
312 higher Olsen-P concentrations related to higher soil-enzyme activity and generally faster cycling of water and
313 elements. This area receives an annual precipitation above 1000 mm, so water is not likely an important
314 limiting factor. All these data thus strongly suggest that N is the limiting nutrient and that invasive plant
315 success depends on a large capacity of N uptake, reduced soil N availability and a higher limitation of N. The
316 current literature suggests that alien invasion in nutrient-rich environments frequently favors plant species
317 with high rates of photosynthesis and growth (Baruch and Goldstein 1999; Leishman et al. 2007; Mozdzer
318 and Zieman 2010; Feng et al. 2011), low costs of foliar construction (Nagel and Griffin 2001; Feng et al.
319 2007; González et al. 2010), large investments of N in photosynthetic production (Ehrenfeld 2003; Xu et al.
320 2007; Feng 2008; Shen et al. 2011), higher capacities of nutrient uptake (Zabinsky et al. 2002; Harrington et
321 al. 2004; Blank and Sforza 2007; Feng 2008; Blank 2010; Hewins and Hyatt 2010; Leffler et al. 2011; Peng et
322 al. 2011) and high levels of plasticity in the acquisition of resources as a function of pulses in nutrient
323 availability (Leffler et al. 2011). These factors indicate that higher efficiency in nutrient uptake and foliar
324 traits enabling rapid rates of growth (Leishman et al. 2007; Zabinsky et al. 2002) will help invading species to
325 succeed when resources are not limited (Bray et al. 2003; Funk and Vitousek 2007; Shah et al. 2009).

326 Similar to our findings, Lichstein et al. (2004) reported that the percentage of soil organic matter was
327 negatively correlated with *Ligustrum* basal area. That study hypothesized that this pattern could be associated
328 with litter quality or with the rapid growth rate in *Ligustrum* and presumably with rapid nutrient uptake. Our
329 results support this hypothesis, because *Ligustrum* has rather low concentrations of N and C in its leaf litter.

330 Higher soil concentrations of extractable K were associated with higher K concentrations in the leaf litter
331 of invasive species and of *Myrsine*. Previous studies have demonstrated that invaded stands have less soil
332 moisture (Aragón et al. 2014), suggesting a higher water uptake with the increasing abundance of invasive
333 species (Gerlach 2000; Levine et al. 2003; Holmes et al. 2005; van Wilgen et al. 2008) that in turn decreases
334 runoff (Dye and Jarmain 2004; Gorgens and van Wilgen et al. 2004; Holmes et al. 2005; van Wilgen et al.
335 2008). Invasive plants can thus prevent the loss of K by increasing water uptake, decreasing runoff and taking
336 up more K. Higher K uptake should be correlated with higher concentrations of K in the litter and, in general,

337 with faster plant-soil-plant K cycling. We have observed a marginally significant higher K concentration in
338 the litter of invaded stands, especially in *L. lucidum* litter, coinciding with the lower soil moisture observed in
339 these stands (Aragón et al. 2014). Importantly, ecosystem-level impacts of an invasive species depend on the
340 combination of traits that determine its per capita effect, together with its abundance (Drenovsky et al. 2012).
341 *Ligustrum* dominance must be taken into account when assessing its potential impacts at community scale
342 (Aragon et al. 2014).
343
344
345 **Effect of invasion on potential soil enzyme activity**
346 The observed increases in phosphatase and β-glucosidase activities reinforce the general results of soil and
347 litter composition, suggesting that plant invasion accelerates nutrient uptake and nutrient-cycling rates. The
348 success of invasive plants in this semi-wet, sub-tropical ecosystem is thus associated with faster water and
349 nutrient cycles, higher soil-enzyme activities, lower concentrations of some nutrients in the soil and with
350 higher levels of nutrients in stand biomasses and faster growth. These results also suggest cascade effects,
351 because higher soil-enzyme activity and higher N uptake can be related to low ammonium:Olsen-P ratios in
352 soils. Lower soil N:P ratios impact soil trophic webs, increasing the abundance of rapidly growing microbial
353 groups (Elser et al. 2003; Fierer et al. 2007), and may be associated with the observed increase in the potential
354 activity of some soil enzymes. Microbes adjust their extracellular release of soil enzymes to maximize the
355 mobilization of substrates rich in their limiting element (Wallenstein and Weintraub 2008: Burns et al. 2013).
356 The higher C:N ratio of the soil in invaded stands is related to the higher levels of potential soil β-glucosidase
357 activity. This enzyme catalyzes the first steps of the hydrolysis of large C-chains and is critical for the further
358 action of enzymes linked to N and P mineralization (Debosz et al. 1999; Stege et al. 2010).Moreover, since β-
359 glucosidase is involved in cellulose catabolism, more abundant and extended litter production in invaded
360 stands (*Ligustrum* is a perennial species which has an extended period of litter fall) (Aragón 2000; Easdale
361 2006) may also explain the higher activity of this enzyme in this type of forest.
362 The maximum potential enzyme activity depends of the density of active enzymes present in soil. Our
363 results thus show a higher density of soil enzymes, consistent with the idea that invaded stands have a higher
364 investment in the production of soil enzymes. Our findings are thus an indication of enzyme concentration
365 and capacity in the soil but not necessarily of their actual activity in the field. As stated above, soil moisture in
366 stands dominated by *Ligustrum* is lower than in native stands (Lichstein et al. 2004; Aragón et al. 2014).
367 Humidity directly affects enzyme activity by affecting hydrolysis and hence subsequently determines nutrient
368 mineralization. Litter decomposition in four species (two natives and two exotics) tended to be lower in stands
369 dominated by *Ligustrum* (Aragón et al. 2014), and the same was true for a standard substrate (leaves of
370 *Populus* sp.) (Fernandez 2012). Consequently, invaded stands appear to invest more in the production of
371 enzymes (that accumulate in the soil) to compensate for the unfavorable environmental conditions. This is
372 particularly important given the higher growth rate of *Ligustrum* and hence its potentially higher demand for
373 resources.
374

375 **Species-specific effects**

376 We have observed clear differences between the native and invasive species and also some differences
377 among the natives. Whereas the litter traits of *Cinnamomum* and *Cupania* clearly differed from those of the
378 invasive species, *Myrsine* shared several characteristics with them. This observation supports previous
379 studies that found some morphological and demographic similarities among *Myrsine*, *Ligustrum* and *Morus*
380 (Easdale et al. 2007; Easdale and Healey 2009). Easdale et al. (2007) measured 19 demographic variables in
381 29 montane tree species of northwestern Argentina, and *Myrsine* was closer in multidimensional space to
382 *Ligustrum* and *Morus* than to *Cinnamomum* and *Cupania*. *Myrsine* and the two exotic species also shared
383 ecomorphological features (structural, biochemical and morphological) such as seed mass, maximum growth
384 rate and foliar P concentration (Easdale 2006). The similarities were especially evident with *Ligustrum*, which
385 also has an overlapping fructification phenology. All these similarities indicate that the morphological and
386 life-history characteristics of *Myrsine* resemble those of the exotics in this study. The invasion of *Ligustrum*
387 in the study area has caused changes in species cover, dominance, diversity and sapling recruitment (Lichstein
388 et al. 2004; Aragón and Morales 2003). Importantly, native species may be affected in different ways. Exotics
389 may specially affect species that share attributes with them and hence potentially have similar requirements
390 (Drenovsky et al. 2012), as perhaps happens with *Myrsine*, whose recruitment and growth is particularly
391 reduced in stands dominated by *Ligustrum* (Bartolucci 2012). In a comparison of native and invaded stands of
392 similar age, *Myrsine* showed a reduction in sapling recruitment of approximately 85% in *Ligustrum* stands,
393 while *Cinnamomum* and *Cupania* were less affected (between 3.5 and 50%). The same trend was found for
394 changes in basal area of adults (an average reduction of 80% in *Myrsine*) (Bartolucci 2012). *Myrsine* has more
395 similar elemental composition and stoichiometry with invasive species than *Cinnamonium* and *Cupania*,
396 which seem to explain the more negative impact over *Myrsine*. The native *Myrsine* would tend to use the
397 resources in the same way than the invasive species and should compete stronger with it.

398 These results agree with those expected under the biogeochemical niche hypothesis (Peñuelas et al. 2008;
399 2010; Sardans and Peñuelas 2013), which claims that elemental composition (nutrient concentrations and
400 their stoichiometric relationships) varies among plant species as a consequence of differential genotypic
401 expression and functioning. This hypothesis predicts that different plant species growing in the same
402 community would tend to have different elemental compositions to reduce the overlap in the use of soil
403 resources and consequently would reduce direct competition. These results are consistent with the
404 "competitive niche exclusion" a basic paradigm of the ecological niche theory (Bonsall et al. 2004; Phillips et
405 al. 2004; Levine and HilleRisLammbers 2009; Alder et al. 2010). In this case the native species with more
406 similar biogeochemical niche to the invasive species was the species first affected by the success of the
407 invasive species. The biogeochemical niche thus appears as a useful tool to detect niche overlap intensity in
408 the study of invasive species, frequently very difficult to be investigated in field conditions (Mooney and
409 Cleland 2001; Davies et al. 2007). Moreover, it can provide clues of which native species would present the
410 highest sensitivity to invasive species spread.

411 In addition, we would like to acknowledge that even though our design intended to control the potential
412 inherent variability between invaded and native stands, we cannot unequivocally assigned the differences in
413 soil enzyme or nutrients to the treatment effect. This is a common and recognized limitation in studies at
414 landscape scale such as most of the studies related to invasive species ecology (van Kleunen et al. 2010). The
415 comparative approach remains, so far, as one of the most commonly used in the field (e.g. , Leishman et al.
416 2010; Tecco et al. 2010). However, these limitations have to be taken into account when interpreting the
417 results.
418
419 **Final remarks and conclusions**
420 Even though several studies have highlighted the role of biological invasions at global scale, invasion
421 ecology still lacks general influential hypotheses (Strayer 2012). Several attempts that intended to identify
422 traits associated with invasion impacts have yield mixed results mainly because successful invasions are
423 closely linked to native community assembly and environmental filters (Drenovsky et al. 2012). In the context
424 of invasion ecology, understanding the link between invasion and ecosystem functioning is crucial to fully
425 evaluate the effect of invasive species. In this study, even though we focused on one particular exotic species,
426 we intended to provide general understanding of the potential effect of this species on the functioning of the
427 ecosystem.
428 Our results indicated that invasion by *Ligustrum* increased the maximum potential of alkaline phosphatase
429 and β-glucosidase activities, probably by compensating for the lower soil water content of the invaded soil, as
430 compared to native forest soil. These observations are supported by previously documented increases in water
431 and plant nutrient uptake in invaded stands (Ayup et al. 2014; Aragón et al. 2014). Plant invasion decreased
432 the availability of soil N, likely the limiting nutrient (litter N:P ratio of 7.4, based on mass) in these soils,
433 whereas it increased the availability of soil P. The lower soil water content and the higher growth capacity of
434 alien plants, coinciding with higher plant nutrient uptake, the large investment in soil-enzyme activity and the
435 lower N and P concentrations in litter, thus suggesting a link between the success of alien plants and a higher
436 capacity to take up nutrients and retain them in the biomass and generally in the ecosystem. Our results
437 showed that the species most sensitive to invasion, *Myrsine*, had a litter composition more similar to that of
438 the invasive plants than to other native plants. This result strongly suggests that this native species and the
439 exotics *Ligustrum* and *Morus* use resources very similarly, and thus *Myrsine* is most directly affected by
440 competitive pressure from invasive species.
441
442
443 **Acknowledgements**
444 This research was supported by the Spanish Government grant CGL2013-48074-P, the Catalan Government
445 project SGR 2014- 274 and the European Research Council Synergy grant ERC-2013-SyG-610028
446 IMBALANCE-P. R. Aragón's stay at CREAF was supported by an external grant from CONICET (National
447 Research Council, Argentina).
448

449 **References**

450 Alder PB, Ellner SP, Levine JM (2010) Coexistence of perennial plants: an embarrassment of niches. Ecology
451 Letters 13: 1019-1029
452 Allison SD, Vitousek PM (2004) Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i.
453 Oecologia 141:162-619
454 Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs.
455 Soil Biol Biochem 37:937 -944
456 Aragón R, Morales JM (2003) Species composition and invasion in NW Argentinean secondary forests:
457 Effects of land use history, environment and landscape. J Veg Sci 14: 195-204
458 Aragón R, Montti L, Ayup MM, Fernández R (2014) Exotic species as modifiers of ecosystem processes:
459 Litter decomposition in native and invaded secondary forests of NWArgentina. Acta Oecologica
460 54:21-28Ayup MM, Montti L, Aragón R , Grau HR (2014) Invasion of *Ligustrum lucidum* (Oleaceae)
461 in the southern Yungas: Changes in habitat properties and decline in bird diversity. Acta Oecologica
462 54: 72-81
463 Bartolucci G (2011) Descripción de los bosques de *Ligustrum lucidum* (Oleácea) en la selva de Yungas en la
464 provincia de Tucumán y su influencia sobre la comunidad de renovales. Undergraduate dissertation.
465 University of Tucumán. Argentina
466 Baruch Z, Goldstein G (1999) Leaf construction cost, nutrient concentration, and net CO2 assimilation of
467 native and invasive species in Hawaii. Oecologia 121: 183-192
468 Bianchi AR, Yañez C (1992) Las precipitaciones del Noroeste Argentino. Instituto de Tecnología
469 Agropecuaria. Argentina.
470 Biondini ME, Bonham CD, Redente EF (1985) Secondary successional patterns in a sagebrush (*Artemisia*
471 *tridentata*) community as they relate to soil disturbance and soil biological activity. Vegetatio 60: 25-
472 36.
473 Blank RR (2010) Intraspecific and interspecific pair-wise seedling competition between exotic annual grasses
474 and native perennials: plant-soil relationships. Plant Soil 326: 331-343
475 Blank RR, Sforza R (2007) Plant-soil relationships of the invasive annual grass *Taeniatherum caput-medusae*:
476 a reciprocal transplant experiment. Plant Soil 298: 7-19
477 Bonsall MB, Jansen VAA, Hassell MP (2004) Life history trade-offs assemble ecological guilds. Science 306:
478 111-114.
479 Bray SR, Kitayima K, Sylvia DM (2003) Mycorrhizae differentially alter growth physiology, and competitive
480 ability of an invasive shrub. Ecol Appl 13: 565-574
481 Brown AD, Grau HR, Malizia LR, Grau A (2001). Argentina. In: Kappelle M and Brown AD (Eds.), Bosques
482 nublados del Neotrópico. Instituto Nacional de Biodiversidad (INBio), Santo Domingo de Heredia,
483 Costa Rica, pp 623-659
484 Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN,
485 Zoppini A (2013) Soil enzymes in a changing environment: Current knowledge and future directions.
486 Soil Biol Biochem 58: 216-234

487 Cabrera AL, Willink A (1980) Biogeografía de América Latina. Organización para Estados Americanos
488 (OEA), Washington, US.
489 Castro- Díez P, Godoy O, Alonso A, Gallardo A, Saldaña A (2013) What explains variation in the impacts of
490 exotic plant invasions on the nitrogen cycle? A meta-analysis. Ecol Lett doi: 10.1111/ele.12197
491 Criquet S, Ferre E, Farnet AM, Le Petit J (2004) Annual dynamics of phosphatase activities in an evergreen
492 oak litter: influence of biotic and abiotic factors. Soil Biol Biochem 36: 1111–1118.
493 Davies KF, Harrison S, Safford HD, Viers JH (2007) Productivity alters the scale dependence of the diversity-
494 invasibility relationship. Ecology 88: 1940-1947
495 Debosz K, Rasmussen PH, Pedersen AR (1999) Temporal variations in microbial biomass C abd cellulolytic
496 enzyme activity in arable soils: effects of organic matter input. Appl Soil Ecol 13: 209-218
497 Drenovsky RE, Grewell BJ, D'Antonio CM, Funk JL, James JJ, Molinari N, Parker IM, Richards CM (2012)
498 A functional trait perspective on plant invasion. Annals of Botany 110: 141-153
499 Dye P, Jarmain C (2004) Water use by black wattle (*Acacia mearnsii*): implications for the link between
500 removal of invaded trees and catchment streamflow response. S Afr J Sci 100: 40-44
501 Easdale TA (2006) Comparative morphologies and life histories amongst montane tree species in North-
502 Western Argentina. PhD Dissertation. University of Wales, Bangor, United Kingdom
503 Easdale TA, Healey JR, Grau HR, Malizia A (2007) Tree life histories in a montane subtropical forest:
504 species differ independently by shade-tolerance, turnover rate and substrate preference. J Ecol 95:
505 1234-1239
506 Easdale TA, Healey JR (2009) Resource-use-related traits correlate with population turnover rates, but not
507 stem diameter growth rates, in 29 subtropical montane tree species. Perspectives in Plant Ecology,
508 Evolution and Systematics 11: 203–218
509 Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of exotic understory
510 plants in deciduous forests. Ecol Appl 11: 1287-1300.
511 Ehrenfeld JG (2003) Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. Ecosystems 6:
512 503–523
513 Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie S, Fagan W, Schade J, Hood
514 J, Sterner RW (2003) Growth rate-stoichiometry couplings in diverse biota. Ecol Lett 6:936-943
515 Evans RD,. Rimer R, Sperry L, Belnap J (2001). Exotic plant invasion alters nitrogen dynamics In an arid
516 grassland. Ecol Appl 11: 1301-1310
517 Feng YL, Auge H, Ebeling SK (2007) Invasive *Buddleja davidii* allocates more nitrogen to its photosynthetic
518 machinery than five native woody species. Oecologia 153: 501-510
519 Feng YL (2008) Photosynthesis, Nitrogen allocation and specific leaf area in invasive *Eupatorium*
520 *adenophorum* and native *Eupatorium japonicum* grown at different irradiances. Physiologia Plantarum
521 133: 318-326
522 Feng YL, Li YP, Wang RF, Callaway RM, Valiente-Banuet A, Inderjit (2011) A quicker return energy-use
523 strategy by populations of a subtropical invader in the non-native range: a potential mechanism for the
524 evolution of increased competitive ability. J Ecol 99: 1116-1123

525 Fernandez RD (2012) Descomposición de hojarasca en Bosques secundarios de la Sierra de San Javier: efecto
526 de las especies exóticas. Undergraduate dissertation. University of Tucumán. Argentina
527 Fierer N, Bradford MA, Jackson RB (2007) Towards an ecological classification of soil bacteria. Ecology
528 88:1354-1364
529 Flory LS, Clay K (2010) Non-native grass invasion alters native plant composition in experimental
530 communities. Biol Inv 12:1285-1294
531 Funk JL, Vitousek PM (2007) Resource use efficiency and plant invasion in low-resource systems. Nature
532 446:1079-1081
533 Gerlach Jr. JD (2000) A model experimental system for predicting the invasion success and ecosystem
534 impacts of non-indigenuous summer-flowering annual plants in California's Central Valley grasslands
535 and oak woodlands. PhD dissertation, University of California, Davis, CA.
536 González AL, Kominoski JS, Danger M, Ishida S, Iwai N, Rubach A (2010) Can ecological stoichiometry
537 help explain patterns of biological invasion? Oikos 119: 779-790
538 Gorgens AHM, Wilgen BW (2004) Invasive alien plants and water resources in South Africa: current
539 understanding, predictive ability and research challenges. S Afr J Sci 100: 27-33
540 Grau HR, Brown AD (1995) Patterns of tree species diversity along latitudinal and altitudinal gradients in the
541 Argentinean subtropical montane forests. In: Churchill SP, Balslev H, Forero E and Luteyn J (Eds.)
542 Biodiversity and conservation of Neotropical montane forest. New York Botanical Garden, Bronx,
543 NY, pp 295-300
544 Grau HR, Arturi MF, Brown AD, Aceñolaza PG (1997) Floristic and structural patterns along a
545 chronosequence of secondary forest succession in Argentinean subtropical montane forests. Forest
546 Ecol Manag 95: 161-171
547 Grau HR, Aragón R (2000) Arboles invasores de la Sierra de San Javier. In: Grau HR and Aragón R (Eds.)
548 Arboles Exóticos de las Yungas Argentinas, LIEY-Universidad Nacional de Tucumán, Tucumán,
549 Argentina, pp 5-20
550 Grau HR, Aide TM (2007) Are Rural–Urban Migration and Sustainable Development Compatible in
551 Mountain Systems? Mt Res Dev 27: 119-123.
552 Grau HR, Hernández ME, Gutierrez J, Gasparri NI, Casavecchia MC, Flores EE, Paolini L (2008) A peri-
553 urban neotropical forest transition and its consequences for environmental services. Ecol Soc 13(1): 35
554 [online] URL: http://www.ecologyandsociety.org/vol13/iss1/art35/
555 Harrington RA, Fownes JH, Cassidy TM (2004) Japanese Barberry (*Berberis thunbergii*) in forest understory:
556 leaf and whole plant reponses to nitrogen availability. Am Midl Nat 151: 206-216
557 Hewins DB, Hyatt LA (2010) Flexible N uptake and assimilation mechanisms may assist biological invasion
558 by *Alliaria petiolata*. Biol Inv 12: 2639-2647
559 Holmes PM, Richardson DM, Elser KJ, Witkowski ETF, Fourie S (2005) A decision-making framework for
560 restoring riparian zones degraded by invasive alien plants in South Africa. S Afr J Sci 101: 553-564

561 Joanisse GD, Bradley RL, Preston CM, Munson AD (2007) Soil enzyme inhibition by condensed litter
562 tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*. New Phytologist
563 175: 535-546
564 Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of
565 ammonium. Biol Fert Soils 6: 68-72
566 Kolb A, Alpert P , Enters D, Holzapfe C (2002) Patterns of invasion within a grassland community. J Ecol
567 90: 871-881
568 Kourtev PS, Ehrenfeld JG, Huang WZ (2002) Enzyme activities during litter decomposition of two exotic and
569 two native plant species in hardwood forests of New Jersey. Soil Biol Biochem 34: 1207-1218
570 Ladd JM, Brisbane PG, Butler JHA (1976) Studies on soil fumigation. 3. Effects on enzyme-actrivities,
571 bacterial numbers and extractable ninhydrin reactive compounds. Soil Biol Biochem 8: 255-260.
572 Leffler AJ, Monaco TA, James JJ (2011) Nitrogen acquisition by annual and perennial grass seedlings: testing
573 the roles of performance and plasticity to explain plant invasion. Plant Ecol 212: 1601-1611
574 Leishman MR, Haslchurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and invasive plants:
575 community-and global-scale comparisons. New Phytologist 176: 635-643
576 Leishman MR, Thomson VP, Cooke J (2010) Native and exotic invasive plants have fundamentally similar
577 carbon capture strategies. J. Ecol 98:28-42
578 Levine JM, Vilà M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the
579 impacts of exotic plants. Proceedings of the Royal Society London *B* 270: 775-781
580 Levine JM, HilleRisLambers J (2009) The importance for the maintenance of species diversity. Nature 461:
581 254-U130.
582 Lichstein JW, Grau HR , Aragón R (2004) Recruitment limitation in secondary forests dominated by an
583 exotic tree. J Veg Sci 15: 721-728
584 McCune B, Mefford MJ (1999) PC-ORD Multivariate analysis of ecological data. Version 3. 0. MjM
585 Software Design, Gleneden Beach, OR.
586 McGroddy M E, Daufresne T, Hedin LO (2004) Scaling of C : N : P stoichiometry in forests worldwide:
587 Implications of terrestrial redfield-type ratios. Ecology 85:2390-2401
588 Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proceedings of the National
589 Academy of Sciences USA 98: 5446-5451
590 Mozder TJ, Zieman JC (2010) Ecophysiological differences between genetic lineages facilitate the invasion
591 of non-native *Phragmites australis* in North American Atlantic coast wetlands. J Ecol 98: 451-458
592 Nagel JM, Griffin KL (2001) Construction cost and invasive potential: comparing *Lythrum salicaria*
593 (*Lythraceae*) with co-occurring native species along pond banks. Am J Bot 88: 2252-2258
594 Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction
595 with sodium bicarbonate. USDA. Cir. n 939.
596 Peng RH, Fang CM, Li B, Chen JK (2011) *Spartina alterniflora* invasion inceases soil inorganic nitrogen
597 pools through interactions with tidal subsides in the Yangtze Estuary, China. Oecologia 165: 797-807

598 Peñuelas J, Sardans J, Ogaya R, Estiarte M (2008) Nutrient stoichiometric relations and biogeochemical niche
599 in coesxisting plant species: effect of simulated climatic change. Pol J Ecol 56, 4: 613-622
600 Peñuelas J, Sardans J, Llusia Owen S, Carnicer J, Giambelluca TW, Rezende EL, Waite M, Niinemets Ü
601 (2010) Faster returns on leaf economics and different biogeochemical niche in invasive compared with
602 native plant species. Global Change Biol 16:2171-2185
603 Phillips RA, Silk JRD, Phalan B, Catry P, Croxall JP (2004) Seasonal sexual segregation in two Thalassarche
604 species: competitive exclusion, reproductive role specialization or foraging niche divergence?
605 Proceedings of the Royal Society B-Biological Sciences 271: 1283-1291
606 Sardans J, Peñuelas J (2005) Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L.
607 forest. Soil Biol Biochem 37: 455-461
608 Sardans J, Peñuelas J, Ogaya R (2008) Experimental drought reduced acid and alkaline phosphatase activity
609 and increased organic extractable P in soil in a *Quercus ilex* Mediterranean forest. Eur J Soil Biol 44:
610 509-520
611 Sardans J, Peñuelas J (2010). Soil enzyme activity in a Mediterranean forest after six Years of drought. Soil
612 Science Society of American Journal 74: 838-851
613 Sardans J, Peñuelas J (2012) The role of plants in the effects of Global Change on nutrient availability and
614 stoichiometry in the plant-soil system. Plant Physiol 160: 1741-1761
615 Sardans J, Peñuelas J (2013). Climate and taxonomy underlie different elemental concentrations and
616 stoichiometries of forest species: the optimum "biogeochemical niche". Plant Ecol 215: 441-455
617 Schinner F, Ohlinger R, Kandeler E, Margesin R (1996) Methods in Soil Biology. Springer Lab Manual,
618 Berlin/Heidelberg/New York/Tokyo
619 Shah MA, Reshi ZA, Khasa DP (2009) Arbuscular mycorryzhas: drivers or passengers of alien plant invasion.
620 Botanical Review 75: 397-417
621 Shen XY, Peng SL, Chen BM, Pang JX, Chen LY, Xu HM, Hou YP (2011) Do higher resource capture
622 ability and utilization efficiency facilitate the successful invasion of native plants? Bio Inv 13: 869-881
623 Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, Repert D, Weiland T (1993) Wood
624 decomposition: Nitrogen and phosphorus dynamics in relation to extracellular enzyme activity.
625 Ecology 74:1586-1593
626 Stege PW, Messina GA, Bianchi G, Olsina RA, Raba J (2010) Determination of b-glucosidase activity in soils
627 with a bioanalytical sensor modified with multiwalled carbon nanotubes. Anal Bioanal Chem 397:
628 1347-1353
629 Strayer DL (2012) Eight questions about invasions and ecosystem functioning. Ecol. Lett 17: 1199-1210
630 Tabatabai MA (1994) Soil enzymes. In: Bottomley PS, Angle JS and Weaver RW (Eds.) Methods of Soil
631 Analysis: Part 2- Microbiological and Biochemical Properties. Soil Science Society of America,
632 Madison, USA, pp 775-833
633 Tecco P, Diaz S, Cabido M, Urcelay C (2010) Functional traits of alien plants across contrasting climatic and
634 land-use regimes: do aliens join the locals or try harder than them?. J. Ecol 98:17-27

635 Tharayil N, Alpert P, Bhowmik PC, Gerard P (2013) Phenolic inputs by invasive species could impart
636 seasonal variations in nitrogen pools in the introduced soils: a case study with *Polygonum cuspidatum*.
637 Soil Biol Bioche 57: 858-867
638 Van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and
639 non-invasive plant species. Ecol. Lett 13: 235-245.
640 Van der Putten WH, Klironomos JN, Wardle DA (2007) Microbial ecology of biological invasions. The
641 ISME Journal 1: 28-37
642 Van Wilgen BW, Reyers B, Le Maitre DC, Richardson DM, Schonegevel L (2008) A biome-scale assessment
643 of the impact of invasive alien plants on ecosystems services in South Africa. J Environ Manage 89:
644 336-349
645 Wallenstein MD, Weintraub MN (2008) Emerging tools for measuring and modeling the in situ activity of
646 soil extracellular enzymes. Soil Biol Biochem 40:2098-2106
647 Xu CY, Griffin KL, Schuster WSF (2007) Leaf phenology and seasonal variation of photosynthesis of
648 invasive *Berberis thunbergii* (Japanese barberry) and two co-occuring native understory shrubs in a
649 northeastern United States deciduous forest. Oecologia 154: 11-21
650 Zabinsky CA, Quinn L, Callaway RM (2002) Phosphorus uptake, not carbon transfer, explains arbuscular
651 mycorrhizal enhancement of *Centaurea maculosa* in the presence of native grassland species. Funct
652 Ecol 16: 758-765
653

654

655 **Fig. 1** Principal component analysis with three sets of variables: enzymes, nutrients and nutrient ratios (20
656 variables) measured in invaded (red) and native (black) stands. The symbols indicate the corresponding score
657 group means ± SE. and the arrows represent variable eigenvectors in the space plotted by the first two PCA
658 axes. The projection of the lines along each axis indicates their relative importance

659

660 **Fig. 2** (a) Principal component analysis with 13 variables (nutrients and nutrient ratios) of the five species
661 studied. C (*Cinnamomum*), Cu (*Cupania*) and My (*Myrsine*) are native species. L (*Ligustrum*) and M (*Morus*)
662 are exotic species (axes 1 and 2). The symbols correspond to the score group means ± SE. Different letters
663 indicate significant differences among species at each axis at $p < 0.05$. (b) Variable eigenvectors in the space
664 plotted by the first two PCA axes. The projection of the lines along each axis indicates their relative
665 importance

666

667 **Fig. 3** (a) Principal component analysis with 13 variables (nutrients and nutrient ratios) of the five species
668 studied. Codes as in Figure 2a (axes 1 and 3). The symbols correspond to the score group means ± SE.
669 Different letters indicate significant differences among species at each axis at $p < 0.05$. When big arrows are
670 present they indicate significant differences between invasive and exotic species. (b) Variable eigenvectors in
671 the space plotted by the first and third PCA axes. The projection of the lines along each axis indicates their
672 relative importance

673

674

675

676