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1 **Litter mixture effects on nitrogen dynamics during decomposition predominantly vary among biomes**
2 **but little with litter identity, diversity and soil fauna**

3

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33

34 **Abstract**

35 Nitrogen (N) is a critical element for net primary production and much of the required N in terrestrial
36 ecosystems is derived from N recycling via litter decomposition. The diversity and identity of plant species
37 and decomposer organisms affect N cycling during litter decomposition, yet the generality and magnitude of
38 these effects remain uncertain. To fill this gap, a decomposition experiment with four leaf litter species that
39 differed widely in initial litter quality was conducted including single species and all possible multispecies
40 mixtures with and without access by microarthropods across a broad latitudinal gradient covering four major
41 forest biomes of the Northern Hemisphere. Furthermore, to quantify mineral N immobilization by microbial
42 decomposers, we labelled the mineral soil underneath the leaf litter with low amounts of ¹⁵N and quantified
43 the transfer and incorporation of the label into each individual litter species from all mixtures at the end of the
44 incubation. The results showed that leaf litter N loss and N transfer from soil into leaf litter in single species
45 treatments depended primarily on litter species identity and the local environmental context. We found strong
46 mixture effects, that overall tended to increase N loss and to reduce ¹⁵N transfer. The relative mixture effects
47 on N dynamics (both N loss and N transfer) differed among forest biomes, but were little affected by the other
48 factors we manipulated. The N loss of individual litter species in mixtures not only depended on litter identity
49 and soil microarthropod access, but also on forest biomes; while ¹⁵N transfer depended strongly on litter
50 mixing, independently of litter species richness or composition of the mixtures. Litter N loss and N
51 immobilization depended strongly on the concentrations of N, calcium and condensed tannins, and the
52 concentrations of calcium, magnesium and condensed tannins, respectively, both in single litter species and
53 mixtures across forest biomes, regardless of species richness and microarthropod access. The results highlight
54 that litter N dynamics primarily depend on leaf litter species identity and can be predicted by a limited set of
55 easily measurable litter traits across a wide variety of biomes. Importantly, the results suggest that soil
56 microarthropods are only of little importance for litter N dynamics across biomes, indicating that
57 microorganisms are the major actors responsible for litter diversity effects on N dynamics in mixtures. To
58 improve predictions on how changes in tree species composition and diversity may impact nutrient dynamics
59 in forest ecosystems in face of increasing N deposition, interactions between litter and soil but also within litter
60 mixtures need closer attention.

61
62 *Keywords:* Nitrogen immobilization; Nitrogen flux; Litter mixture; Stable isotopes; Litter diversity; Litter
63 quality; Litter traits; Soil fauna; Microarthropods; Plant litter-soil interactions.

64

65 **1. Introduction**

66 Human activities result in the loss of biodiversity, which can alter ecosystem functions ^{1, 2, 3}. Past
67 research typically measured aboveground plant biomass production as one variable of ecosystem functioning
68 and its dependence on plant-species richness ^{4, 5, 6}. Experiments have shown that primary productivity is
69 positively related to plant-species diversity worldwide both in grassland and forest ecosystems ^{3, 7, 8}. However,
70 much less is known about how biodiversity affects other key ecosystem processes, such as decomposition and
71 nutrient cycling ^{4, 9, 10}.

72 The frequently observed diversity effects on the decomposition of mixed plant litter ^{11, 12} are based on
73 a number of non-exclusive mechanisms resulting in higher or lower decomposition rates and nutrient release
74 rates of litter mixtures compared to monospecific litter ^{9, 13, 14}. Even if the litter mixture as a whole does not
75 show any difference compared to the predicted decomposition rates based on monospecific litter, individual
76 litter species in the mixtures may be affected in opposite ways. Possible mechanisms underlying such mixture
77 effects include nutrient transfer between different litter species with different decomposability ^{9, 15}, altered
78 decomposer activity by specific litter characteristics ^{16, 17} and positive feedback of decomposers resulting from
79 complementary resource use and higher habitat availability ^{12, 18, 19}.

80 Nitrogen (N) transfer between decomposing litter of chemically distinct litter species has been
81 reported in previous studies ^{20, 21} and identified as powerful mechanism responsible for litter mixture effects,
82 given that N is typically a rate limiting factor at an early stage of decomposition. Indeed, compared to the
83 stoichiometry of the body tissue of decomposers, fresh plant litter is poor in N ^{14, 22, 23}. Consequently,
84 decomposer organisms may be limited by N during early stages of litter decomposition, and they take up N
85 from external sources (typically the underlying soil) resulting in litter N accumulation ^{21, 24, 25}, a process known
86 as N immobilization ²⁶. Later in the decomposition process, when litter C-to-N ratios reach lower values,
87 typically between 55 ²⁷ and 40 ²⁸, decomposers release N as NH₄. This net N release, however, underestimates
88 total litter N release because N losses are in part counterbalanced by the incorporation of exogenous N during
89 litter decomposition ^{29, 30, 31}. These bi-directional N fluxes are more difficult to study and are much less
90 understood than carbon fluxes and the commonly used mass balance of total litter mass.

91 Litter mixture effects play an important role in regulating N immobilization and mineralization ^{20, 32, 33}.
92 Nitrogen dynamics during litter decomposition can impact plant performance and plant community
93 composition ²⁸, which in turn modulates ecosystem N cycling by affecting the quality and quantity of plant

94 litter ³⁴. Litter mixture effects on N dynamics can differ considerably from those on carbon or mass loss
95 dynamics ^{14, 21, 35}. For example, complementarity effects in leaf litter mixtures were found to be considerably
96 stronger on N loss than on carbon loss ^{14, 36}. As one of the key roles in the decomposition triangle concept, i.e.
97 the interplay between environment, litter characteristics and decomposer community, the importance of soil
98 fauna for litter decomposition and N dynamics has long been recognised ^{11, 37}. A recent global meta-analysis
99 found the soil macrofauna to increase litter mass loss on average by 40% ¹¹, but soil mesofauna was estimated
100 to increase litter N loss by only about 2% across ecosystems and latitudes ³⁶. Furthermore, litter mixtures and
101 soil fauna are likely to interactively affect litter decomposition as litter species diversity affects litter quality,
102 which, in turn, affects soil fauna feeding behaviour ^{13, 19}. However, as environmental context, decomposer
103 communities and litter species all vary among individual studies, the generality of mechanisms responsible for
104 non-additive litter mixture effects on N cycling is difficult to assess, in particular as only few studies followed
105 N dynamics in litter mixtures in detail.

106 Here, we investigated if N loss from decomposing litter and N immobilization in leaf litter depended on
107 initial leaf litter characteristics, leaf litter diversity (i.e., richness or composition of litter species in mixtures)
108 and environmental conditions (i.e., climate, soil biota, soil chemical and physical properties). We conducted a
109 large field experiment using four leaf litter species of common temperate forest tree species (i.e., alder, holly,
110 beech and grey willow) differing widely in initial litter quality ¹⁷. Litter was incubated in single species and all
111 possible multispecies mixtures across a latitudinal gradient covering four major biomes of the Northern
112 Hemisphere, including the subarctic, the boreal, the temperate and the Mediterranean biome. Holding litter
113 quality constant across all biomes, we investigated the relative importance of leaf litter identity, diversity (i.e.,
114 richness or composition of litter species in mixtures) and the decomposer food web composition (i.e., presence
115 or absence of microarthropods) as drivers of N loss from decomposing litter and N immobilization in leaf litter
116 under variable environmental conditions across Europe. We used microcosm-like litter enclosures in the field
117 with two different mesh sizes to manipulate access by microarthropods to evaluate the importance of the impact
118 of soil microarthropods in driving litter mixture effects on N dynamics during litter decomposition. This
119 allowed testing the generality of litter mixing effects under variable environmental conditions and decomposer
120 food web structure independently from differences in site-specific litter identity and quality. To quantify
121 mineral N immobilization by decomposer organisms within the leaf litter we labelled the mineral soil
122 underneath the microcosms with low amounts of a ¹⁵N tracer and followed the transfer and incorporation of

123 the label into each of the four litter species separately in all mixtures by the end of the experiment. We
124 hypothesized that (1) N loss increases and N immobilization decreases in litter mixtures compared to single
125 litter species, because of N transfer processes among litter types of different quality^{20,36} that both stimulate N
126 release and demand less external N transferred from the underlying soil; (2) N dynamics can be predicted from
127 a common set of initial litter characteristics across biomes due to strong litter trait-based effects on
128 decomposition rates^{17,38,39}; and (3) soil microarthropods amplify litter species identity and mixture effects on
129 N dynamics in similar ways across biomes irrespective of species identity and non-additive mixture effects.

130 **2. Materials and Methods**

131 **2.1 Study sites and leaf litter material**

132 Our field experiment followed an identical protocol at four different sites, all in forests encompassing a
133 large latitudinal gradient (about 3,000 km) in the Northern Hemisphere. The sites included subarctic (Sweden),
134 boreal (Sweden), temperate (The Netherlands) and Mediterranean (France) forests described in more detail in
135 Zhou et al.¹⁷. Four different leaf litter species of common temperate forest trees were chosen including alder
136 (*Alnus glutinosa* (L.) Gaertn.), holly (*Ilex aquifolium* L.), grey willow (*Salix cinerea* L.) and beech (*Fagus*
137 *sylvatica* L.) differing considerably in chemical and physical characteristics. For example, N concentrations as
138 an important driver of N dynamics varied from 2.49 ± 0.08 % (*A. glutinosa*) to 1.69 ± 0.07 % (*I. aquifolium*)
139 to 1.23 ± 0.07 % (*S. cinerea*) and to 1.03 ± 0.06 % (*F. sylvatica*). The 20 chemical and physical litter
140 characteristics [including C, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na),
141 water soluble carbon (WSC), cellulose (Cell), hemicelluloses (Hcell), lignin (Lig), soluble phenolics (S-Phen),
142 total phenolics (T-Phen), condensed tannins (Tan) and water saturation capacity (Wsat), tri-dimensionality
143 (3D), specific leaf area (SLA), leaf toughness (Tough; g H₂O), tensile strength (Ten) and pH, in addition to N]
144 were determined in an earlier study conducted at the same sites and using the same litter material adopting a
145 community-weighted mean approach to determine litter characteristics of mixtures¹⁷.

146 Leaf litter was collected in autumn 2006 at the temperate forest site in the Netherlands using litter traps
147 placed in the field during leaf fall. Freshly fallen senesced leaf litter were collected every second day, whereas
148 leaf litter of *I. aquifolium* was obtained by cutting branches in the field and simulating senescence by air-drying
149 and allowing laves to fall off from the branches in the laboratory during a period of three to four weeks^{17,36,}
150 ³⁸. The N concentration of *I. aquifolium* leaves collected from branches did not significantly differ from
151 senescent leaves which are shed in the field⁴⁰. After collection, the litter was dried at 40°C for one week.

152 Leaves with signs of herbivory, fungal attack or galls were excluded. Leaf litter from each species was pooled
153 and homogenized.

154 **2.2 Experimental design**

155 The experiment was set up at all four forest sites in autumn 2008. Leaf litter mixtures were prepared in
156 the laboratory and transported in individual plastic bags to the respective field sites. In total there were 15
157 different litter treatments, including four single species (monocultures) and all possible combinations of 2-
158 species (six), 3-species (four), and 4-species (one) mixtures. The leaf litter was exposed in the field using
159 custom-made microcosm-like litter enclosures constructed of polyethylene tubes (height 90 mm, diameter 150
160 mm) and covered with 50 μm mesh at the bottom and a lid covered with 50 μm mesh on top to prevent entry
161 of extraneous litter while allowing water to pass. Two windows (50 x 180 mm) were cut at the side of the
162 microcosms and covered either with 50 μm (only microorganism and microfauna access) or 1 mm mesh that
163 additionally allowed access by microarthropods ^{17, 36}. We chose to use microcosms rather than litterbags to
164 avoid compression of litter that can alter microclimatic conditions and faunal activity. We filled each
165 microcosm with 8 g dry weight leaf litter with mixtures containing equal amounts of each litter type. At each
166 forest site we installed 90 microcosms (15 litter treatments \times 2 mesh sizes \times 3 replicates) arranged in three
167 randomized blocks, resulting in a total of 360 microcosms and an overall replication of 12 microcosms per
168 treatment combination. Microcosms were separated from each other by at least 50 cm and randomly distributed
169 within blocks spaced at least 20 m. Before microcosms were installed, the local litter layer underneath the
170 microcosms was carefully removed and the mineral soil was labeled with ¹⁵N by adding 10 mg ¹⁵NH₄¹⁵NO₃
171 (99 atom% ¹⁵N; Campro Scientific, Berlin, Germany) in 100 ml distilled water. Microcosms were then placed
172 on top of the labeled soil surrounded by the local litter layer providing decomposers access (through the lateral
173 windows of microcosms) to a continuous layer of litter.

174 After approximately one year in the field, microcosms were retrieved; the exact duration of litter
175 decomposition varied somewhat among biomes, for details see Zhou et al. ¹⁷. Microcosms were opened on site,
176 litter was removed and transported in individual plastic bags to the laboratory. Litter mixtures were sorted to
177 component species to assess the N loss of individual litter species in mixtures. Attached mineral material was
178 brushed off and litter was dried at 65°C for two days. For stable isotope analysis, dried litter was ground to
179 powder and isotope ratios (¹⁵N/¹⁴N) were measured with an elemental analyser (NA 1500, Carlo Erba, Milan,

180 Italy) and an isotope ratio mass spectrometer (MAT 251, Finnigan, Bremen, Germany). Nitrogen in air was
181 used as a standard and acetanilide (C₈H₉NO, Merck, Darmstadt, Germany) for internal calibration.

182 **2.3 Calculations and statistical analysis**

183 Two blocks, one at the boreal and one at the temperate site, had to be dropped from the analyses due to
184 destruction of microcosms by wild animals, resulting in a total of 300 intact microcosms at harvest ¹⁷. Net
185 changes in the amount of leaf litter N (N loss) during decomposition were calculated as:

$$186 \text{ N loss (\%)} = \frac{M_0 \times N_0 - M_e \times N_e}{M_0 \times N_0} \times 100$$

187 with M_0 and M_e the initial and final dry weight of individual litter species, respectively, and N_0 and N_e the
188 initial and final N concentration.

189 Transfer of ¹⁵N into litter samples was calculated by subtracting natural ¹⁵N atom% in the initial litter
190 from ¹⁵N atom% at the end of the experiment.

191 To detect any non-additive mixture effects on litter N loss and ¹⁵N transfer, we calculated the relative
192 mixture effect (RME) for each mixture as:

$$193 \text{ RME (\%)} = \frac{m_{obs} - m_{exp}}{m_{exp}} \times 100$$

194 with m_{obs} the observed N loss or ¹⁵N transfer of the litter mixture and m_{exp} the expected N loss or ¹⁵N transfer
195 of the same litter mixture calculated as mean N loss or ¹⁵N transfer of the component litter species decomposing
196 in single litter species microcosms at each site ⁴¹. Deviations from zero indicated non-additive mixture effects
197 with positive and negative values referred to as synergistic and antagonistic effects, respectively ⁴².

198 To explore the mechanisms underlying non-additive effects in litter mixtures, we compared individual N
199 loss and ¹⁵N transfer of litter species in mixtures to the average N loss and ¹⁵N transfer of single litter species
200 and calculated relative individual performance as:

$$201 \text{ RIP (\%)} = \frac{m_{obs_mix} - m_{obs_sing}}{m_{obs_sing}} \times 100$$

202 with m_{obs_mix} the observed N loss or ¹⁵N transfer of individual litter species in mixtures and m_{obs_sing} the observed
203 N loss or ¹⁵N transfer of the same single litter species at each site ⁴¹.

204 Variations in leaf litter N loss (% of initial, log transformed) and ¹⁵N transfer (μg ¹⁵N g⁻¹ dry weight
205 litter, log transformed) into leaf litter species incubated in single species were analyzed with full factorial
206 ANOVAs (GLM; type III sum of squares) with the factors forest biome (FB; subarctic, boreal, temperate and

207 Mediterranean) treated as random factor, and litter species identity [SI; *A. glutinosa* (A), *I. aquifolium* (I), *S.*
208 *cinerea* (S), and *F. sylvatica* (F)] and access by microarthropods (M; access or no access) as fixed factors.

209 Analysis of variance models based on sequential sums of squares (type I) were used to assess the effects
210 of FB, M, litter species richness (SR; 2, 3 and 4 species), litter species composition (SC; AI, AS, IAS, AF,
211 FIA, ASFI, ASF, SI, FI, SFI and SF) and interactions of FB × SR, FB × SC, FB × M, M × SR, M × SC, FB ×
212 SR × M and FB × SC × M on RME, annual litter N loss and ¹⁵N transfer of whole litter mixtures. A similar
213 model (ANOVA; type I sum of squares) with FB, SI, SR, SC and M as main factors and interactions of FB ×
214 SI, FB × SR, FB × SC, FB × M, SI × SR, SI × SC, SI × M, M × SR, M × SC and FB × SI × M was used to
215 analyze variations in annual N loss and ¹⁵N transfer into individual litter species (RIP) in mixtures. In addition,
216 we used Student's t tests to inspect if RIP of litter species and RME on annual litter N loss and ¹⁵N transfer
217 significantly differed from zero and between soil community treatments.

218 We used partial least square (PLS) regressions to assess the relative importance of initial chemical and
219 physical leaf litter traits for leaf litter N dynamics (N loss and ¹⁵N transfer) incubated in single litter species
220 and litter mixtures. We used PLS regressions instead of multiple linear regressions because PLS regression is
221 based on the linear conversion of a large number of predictors to a small number of orthogonal factors, thereby
222 reducing multi-collinearity between predictors ⁴³. First, we included the complete set of 20 chemical and
223 physical litter traits as predictors in the regression analysis. Then, a reduced set of litter traits with the highest
224 explanatory power in the full model were used to investigate if litter N dynamics can be explained by a
225 consistent set of traits. The relative importance of individual predictors in the model was estimated by the
226 variable of importance of projection (VIP), with VIP > 1 indicating significant contribution of predictors to
227 variations in the dependent variable ^{42,44}.

228 Prior to statistical analyses, data were inspected for homogeneity of variance using Levene test and
229 log(x+1) transformed if necessary. Data on litter N loss and ¹⁵N transfer were log transformed prior to the
230 analysis. Analyses of variance and comparisons of means (Tukey's HSD test, *P* < 0.05) and Student's t tests
231 were performed using SPSS (Version 27.0.0; SPSS Inc., Chicago, IL, USA). PLS regressions were performed
232 using TANAGRA 1.4.48 ⁴⁵.

233 3. Results

234 3.1 N loss and immobilization in individual litter species

235 After about one year in the field, litter N loss of single litter species ranged from -7 % (i.e., a net N
236 increase of 7 %) for *F. sylvatica* in the boreal forest to 36 % (i.e. a net N decrease of 36 %) for *I. aquifolium*
237 in the temperate forest (Fig. 1a). Species identity explained 37 % of the variance in litter N loss (Table 1a).
238 Averaged across forest biomes and microarthropod treatments, litter N loss increased in the order *F. sylvatica*
239 (-2 %) < *S. cinerea* (5 %) < *I. aquifolium* (13 %) < *A. glutinosa* (18 %; Fig S1a). However, the interspecific
240 differences depended on the site of incubation as indicated by the significant FB × SI interaction explaining
241 an additional 19 % of the variance (Table 1a, Fig. 1a), but were independent of microarthropod access. In
242 particular the loss of N from *I. aquifolium* litter varied among biomes and was very high in the temperate forest
243 but not existing in the boreal forest.

244 Transfer of external N into individual litter species, quantified by the added ¹⁵N, ranged from 9.6 μg g⁻¹
245 dry weight in *F. sylvatica* incubated in the subarctic forest to 58.1 μg g⁻¹ dry weight in *I. aquifolium* incubated
246 in the boreal forest (Fig. 1b). Species identity was of major importance and explained 53 % of the variation in
247 ¹⁵N transfer in individual litter species (Table 1b). Averaged across forest biomes and microarthropod
248 treatments, ¹⁵N transfer into litter species increased in the order *F. sylvatica* (14.8 μg g⁻¹ dry weight) < *S.*
249 *cinerea* (20.8 μg g⁻¹ dry weight) < *A. glutinosa* (31.6 μg g⁻¹ dry weight) < *I. aquifolium* (46.6 μg g⁻¹ dry weight;
250 Fig S1b). Forest biome explained an additional 22 % of the variation in ¹⁵N transfer, mostly driven by the
251 lower ¹⁵N transfer in the subarctic (average of 18.5 μg g⁻¹ dry weight) compared to the other three forest biomes
252 which did not differ significantly (average of 33.0 μg g⁻¹ dry weight). Litter species identity and forest biome
253 differences were not independent as indicated by the significant FB × SI interaction, but the patterns were
254 generally consistent across biomes with the interaction accounting for only 5 % of the variation and
255 microarthropod access being generally not significant (Table 1b, Fig. 1b).

256 3.2 N loss and immobilization in mixtures

257 Litter N loss in litter mixtures varied significantly with species composition explaining 15 % of the
258 variance, but the effect of species composition depended on forest biome with the interaction explaining an
259 even higher, additional 19% of the variation (Table 2a). It ranged from 2 % for the two-species *Salix-Fagus*
260 mixture incubated in the temperate forest to 32 % for the two-species *Alnus-Ilex* mixture incubated in the
261 temperate forest (Fig. 2a). Averaged across biomes litter N loss increased from *Salix-Fagus* (6 %) to *Alnus-*

262 *Ilex* two species mixtures (20 %; Fig S2a). Generally, litter N loss was neither affected by species richness nor
263 by the access of microarthropods.

264 In contrast to N loss, most of the variation in ^{15}N transfer into litter mixtures was explained by forest
265 biome as main effect (37 % of total; Table 2b). Averaged across litter compositions and microarthropod
266 treatments, ^{15}N transfer differed significantly between the subarctic ($13.9 \mu\text{g g}^{-1}$ dry weight) and the other three
267 forest biomes (average of $29.2 \mu\text{g g}^{-1}$ dry weight) (Fig. S3b). Overall, the transfer of the added ^{15}N into litter
268 mixtures ranged from $5.6 \mu\text{g g}^{-1}$ dry weight in the two-species *Salix-Fagus* mixtures incubated in the subarctic
269 forest to $43.0 \mu\text{g g}^{-1}$ dry weight in the two-species *Alnus-Ilex* mixtures incubated in the temperate forest (Fig.
270 2b). In addition to forest biome, ^{15}N transfer into litter mixtures varied significantly with litter species
271 composition which explained 19 % of the variation in the transfer of ^{15}N (Table 2b). Averaged across forest
272 biomes and soil microarthropod treatments, ^{15}N transfer into litter was lowest in the two-species *Salix-Fagus*
273 mixture ($14.2 \mu\text{g g}^{-1}$ dry weight) and highest in the two-species *Alnus-Ilex* mixture ($33.9 \mu\text{g g}^{-1}$ dry weight;
274 Fig. S2b). Neither litter species richness, nor access by microarthropods significantly affected ^{15}N transfer into
275 litter mixtures.

276 3.3 Litter mixture effects on N dynamics

277 Overall, mixture effects on N loss tended to be positive, whereas they tended to be negative on ^{15}N transfer
278 (Fig. 3). The relative mixture effects on N dynamics (both N loss and N immobilization) differed among forest
279 biomes, but were relatively little affected by the other factors we manipulated (Table 3a). In the boreal forest
280 N loss did not deviate from that expected from litter species in monoculture, whereas in the subarctic and the
281 Mediterranean forest, mixtures showed significant synergistic non-additive effects, but in the temperate forest
282 significant (less pronounced) antagonistic non-additive effects (Fig. 3a). Transfer of ^{15}N into the litter also
283 differed mostly among forest biomes, but with overall predominant antagonistic mixture effects (i.e., less
284 immobilization in mixtures than predicted from monocultures) in subarctic, boreal and Mediterranean forests,
285 and no mixture effect in the temperate forest (Table 3b, Fig. 3b). Moreover, there was a significant interaction
286 between forest biome and access by microarthropods, showing that N immobilization was distinctively
287 modified by microarthropods depending on forest biome. In the subarctic and Mediterranean forest, access by
288 microarthropods enhanced antagonistic non-additive mixture effects, whereas in the boreal forest the
289 antagonistic non-additive mixture effect increased when microarthropods had no access (Fig. S5b).

290 3.4 Litter mixture effects on N dynamics of individual litter species

291 Litter mixture effects on N dynamics of individual litter species differed among forest biomes, which
292 also depended on litter species identity and the access by microarthropods (three-way interactions for both N
293 loss and N immobilization; Table 4). Averaged across forest biomes and microarthropod treatments, N loss in
294 mixtures was lower than in the respective monocultures in *F. sylvatica* and *S. cinerea* (mean RIP = -12.7 %
295 and mean RIP = -27.0 %, respectively), but higher in *I. aquifolium* and *A. glutinosa* (mean RIP = +29.9 % and
296 mean RIP = +34.7 %, respectively; Fig. S4a). As indicated by the significant FB × SI × M interaction, this
297 effect varied across forest biomes and microarthropod treatments (Table 4a). The average mixture effect on
298 individual species N loss was significantly lower in the subarctic and temperate forest (mean RIP = -69.1 %
299 and mean RIP = -28.5 %, respectively), but significantly higher in the Mediterranean forest (mean RIP =
300 +109.84%) and not affected in the boreal forest (mean RIP = -1.5 %; Fig S5a). Although significant,
301 modifications by microarthropods were minor and restricted to the Mediterranean biome.

302 Variations in the transfer of ¹⁵N into individual litter species in mixtures were not significantly affected
303 by litter species richness nor by access by microarthropods, but were strongly affected by litter species identity
304 and composition as well as forest biomes (Table 4b). Compared to single species incubations, in mixtures
305 averaged across forest biomes and microarthropod treatments, significantly less ¹⁵N was transferred into *F.*
306 *sylvatica* (mean RIP = -24.9 %), *A. glutinosa* (mean RIP = -20.6 %) and *I. aquifolium* (mean RIP = -14.7 %),
307 whereas significantly more ¹⁵N was transferred into *S. cinerea* (mean RIP = +13.7 %; Fig. S4b). Further, the
308 transfer of ¹⁵N into individual litter species in mixtures varied with forest biome (Table 4b). The average
309 mixture effect on the transfer of ¹⁵N into individual species was significantly lower in the subarctic (mean RIP
310 = -24.0%), boreal (mean RIP = -18.2 %) and Mediterranean forest (mean RIP = -9.3%), but significantly higher
311 in the temperate forest (mean RIP = +10.1 %; Fig S5b). The impact of microarthropods was minor, with the
312 interactions with forest biome and litter species identity being primarily due to a positive effect of soil
313 microarthropods on the ¹⁵N transfer into *S. cinerea* in the Mediterranean biome and negative effects on the ¹⁵N
314 transfer into *F. sylvatica* in the temperate and Mediterranean biome (Fig. S2).

315 3.5 Litter characteristics and N dynamics

316 In single litter species and litter mixtures the set of predictors explaining interspecific variations in litter
317 mass loss differed between forest biomes, but initial concentrations of N, Ca, Mg and condensed tannins in the
318 litter were among the best predictors of mass loss across forest biomes (Table S2). Regression models using

319 only three of these four leaf litter characteristics explained more variation in litter N loss (N, Ca and condensed
320 tannins) and ¹⁵N transfer (Ca, Mg and condensed tannins) in monoculture than in mixture, i.e., 21% vs 5% (N
321 loss) and 65% vs 25% (¹⁵N transfer) in the subarctic forest, 70% vs 10% (N loss) and 58% vs 47% (¹⁵N transfer)
322 in the boreal forest, 45% vs 26% (N loss) and 77% vs 33% (¹⁵N transfer) in the temperate forest as well as
323 50% vs 25% (N loss) and 71% vs 35% (¹⁵N transfer) in the Mediterranean forest (Table 5).

324 **4. Discussion**

325 **4.1 Litter mixture effects on N release**

326 In contrast to our first hypothesis, relative mixture effects on the loss of N from litter only varied
327 significantly among forest biomes suggesting that non-additive effects on litter N loss depend on
328 environmental context, but were little affected by the other factors we manipulated. By contrast, forest biome
329 and litter species identity explained a significant fraction of the variation in N loss in single species in mixtures,
330 but these effects varied significantly between microarthropod treatments, indicating that N loss of individual
331 litter species in mixtures were not only determined by species-specific litter properties within mixtures and
332 microarthropod access, but also depended on environmental context.

333 In contrast to litter mass loss ¹⁷, non-additive effects on litter N loss only depended on environmental
334 context (forest biome), regardless of litter species diversity or access by microarthropods. This highlights that
335 litter mixture effects on N release are mediated by environmental context, which contrasts with previous
336 studies reporting litter diversity effects on decomposition to be mediated predominantly by species identity ¹⁴,
337 ¹⁷. The loss of N did not deviate from that expected from the component litter species in monoculture in the
338 boreal forest, whereas mixtures showed significant synergistic non-additive effects in the subarctic and
339 Mediterranean forest and (minor) significant antagonistic non-additive effects in the temperate forest. The
340 antagonistic effects in the temperate forest may be related to the fact that the litter species used originated from
341 the temperate biome and therefore the decomposer community may have been adapted to respond to variations
342 in the characteristics of these litter species ^{46, 47, 48}. However, the antagonistic effects in the temperate forest
343 and the synergistic non-additive effects in the subarctic and Mediterranean forest may also be related to the
344 decomposition stage ^{9, 49} since we harvested the litter at all sites after a fixed period of time, i.e., 49 to 51
345 weeks. The average mass loss in the temperate forest in single litter species and litter mixtures across
346 microarthropod treatments was 43%, considerably exceeding that in the other sites which were still in at an
347 early stage of decomposition with respective mass loss rates of 24% for subarctic and 34% for both boreal and

348 Mediterranean biome ¹⁷. Previous studies reported that synergistic effects dominated at early stages of
349 decomposition and disappeared ⁵⁰ or switched to antagonistic effects at later stages ⁴². A recent meta-analysis
350 also emphasized that at early stages of decomposition N immobilization by microbes beside litter N itself may
351 explain variations in mixture effects on N release ⁹. According to these findings, we also found the N loss of
352 different litter species to vary strongly in litter mixtures, independently of litter species richness or composition
353 of the mixtures. Rather, access to alternative N sources depended on forest biome and the access of the litter
354 by microarthropods. Averaged across forest biomes and microarthropod treatments, the N loss in mixtures was
355 reduced in *F. sylvatica* and *S. cinerea*, but increased in *I. aquifolium* and in particular in *A. glutinosa* compared
356 to single species incubations. This suggests that decomposers preferentially process more easily decomposable
357 litter species if there is a choice in litter mixtures ^{9, 12}. In fact, the differences mainly resulted from slower
358 decomposition of the two slow decomposing species *F. sylvatica* and *S. cinerea* in mixtures, and from faster
359 decomposition of the two more rapidly decomposing species *A. glutinosa* and *I. aquifolium*.

360 **4.2 Litter mixture effects on N immobilization**

361 In contrast to our first hypothesis, relative mixture effects on ¹⁵N transfer differed among forest biomes
362 and varied with microarthropod access to the litter, suggesting that non-additive effects on litter N
363 immobilization are not only determined by species-specific litter properties within mixtures, but also depend
364 on environmental context including the local soil fauna community. The ¹⁵N transfer into litter species in
365 mixtures differed strongly in their response to litter mixing independently of litter species richness or
366 composition of the mixtures. The strong variation in N immobilization with environmental context is consistent
367 with earlier studies ^{14, 51} and stresses that non-additive effects on litter N immobilization not only depend on
368 specific properties of litter species within mixtures.

369 The significant antagonistic non-additive litter mixture effects on ¹⁵N transfer averaged across forest
370 biomes and microarthropod treatments indicate that less external N was transferred from the soil into litter
371 mixtures than into single litter species. Presumably, this reflects that litter mixtures alleviate initial N limitation
372 compared to single litter species, thus reducing the dependence of decomposer organisms on external N due
373 to N transfer among litter species within mixtures ^{20, 21}. Supporting this conclusion, Lummer et al. ²¹ found
374 increased N transfer between leaf species with increasing difference in leaf N concentrations. Among forest
375 biomes antagonistic mixture effects on the ¹⁵N transfer (i.e., less immobilization in mixtures than predicted
376 from monocultures) were most pronounced in the subarctic, boreal and Mediterranean biome, but non-existing

377 in the temperate biome. As in N loss, differences between forest biomes in the transfer of N into litter may
378 have been due to constraints of decomposer communities to decompose local litter as well as to different stages
379 of decomposition at the different forests with the immobilization of N being of particular importance at early
380 stages of decomposition ^{28, 52}. However, in our experiment, variations in the transfer of ¹⁵N into litter species
381 was independent of litter species richness, composition of the mixtures and microarthropod access, rather, it
382 only depended on litter species identity. This indicates that litter species in mixtures interacted little and that
383 non-additive mixture effects were due to reduced N immobilization in litter species in mixtures. Specifically,
384 the transfer of N into litter of *A. glutinosa*, *F. sylvatica* and *I. aquifolium* was strongly reduced in mixtures,
385 whereas it was increased in *S. cinerea*, although only little. As the effects were independent of the access of
386 litter by microarthropods, the reduced N transfer into litter species in mixtures may reflect a reduced need of
387 microorganisms in mixtures for external N. In fact, litter mixtures may reduce the requirement of
388 microorganisms for importing N from the soil due to more N being accessible in mixtures ^{4, 53, 54}. Differences
389 in microbial biomass and community composition may have contributed to the reduced N demand of
390 microorganisms in mixtures compared to single litter species ^{55, 56, 57}. However, few studies have investigated
391 effects of mixing leaf litter species on microbial community composition or microbial activity ⁵⁸. Interestingly,
392 the biomass of saprotrophic fungi, the main actors of N transfer in decomposing leaf litter, has been shown to
393 increase with increasing leaf litter diversity ⁵⁹.

394 **4.3 Litter identity and N dynamics**

395 We found a net release of N in virtually all single litter species and mixtures, however, in single litter
396 species N loss and ¹⁵N transfer of the four different litter species differed markedly, with the differences in
397 both N loss and ¹⁵N transfer being consistent across forest biomes. Moreover, across forest biomes and soil
398 microarthropod treatments, litter N loss and ¹⁵N transfer in litter mixtures strongly depended on the
399 composition of litter species in mixtures. Overall, the results indicate that leaf litter N dynamics in single litter
400 species as well as mixtures primarily depend on leaf litter species identity and forest biome. Moreover, in line
401 with our second hypothesis, we identified two sets of easy to measure litter characteristics, i.e., concentrations
402 of N, Ca and condensed tannins for N loss, and Ca, Mg and condensed tannins for N immobilization, explaining
403 most of the variation in N loss and immobilization both in single litter species and litter mixtures across forest
404 biomes and large latitudinal gradients.

405 The net N release pattern in single litter species and mixtures support the conclusion of Parton et al. ²⁸
406 that net N release from litter only occurs if the C-to-N mass ratio of the litter is below 40. Only the single
407 species and mixtures of *F. sylvatica* did not show N release and *F. sylvatica* had the lowest initial C-to-N mass
408 ratio of 46.6. This further underpins the key importance of species identity for N dynamics ^{21, 38, 53, 60}. However,
409 the significant interactions between litter species identity or species composition and forest biome suggest that
410 differences in the net N release between litter species were modified by site-specific conditions. Nevertheless,
411 the overall strong litter identity effect points to initial litter quality as predominant driver for N dynamics during
412 litter decomposition with local environmental conditions and decomposer communities being only of
413 secondary importance supporting earlier conclusions ^{28, 38, 60}.

414 Supporting our second hypothesis, variability in N loss and ¹⁵N transfer was best explained by regression
415 models including initial concentrations of Ca and condensed tannins both in single litter species and mixtures.
416 Positive correlations between initial Ca concentration and decomposition have been reported previously and
417 assumed to reflect the important role of Ca in regulating soil pH and macrofauna activity ^{38, 61}. In our study,
418 however, macrofauna access was excluded and the results therefore indicate that litter Ca concentrations also
419 favorably affect microorganisms and microarthropods. Condensed tannins are among the most abundant
420 secondary metabolites of plants and play an important role in plant-herbivore ⁶² as well as litter-detritivore
421 interactions ^{58, 63, 64}. Moreover, condensed tannins can change the composition of microbial communities and
422 have been shown to hamper microbial activity and N dynamics ^{65, 66, 67}. The positive correlation of ¹⁵N transfer
423 into litter and initial litter Mg concentrations presumably is due to the important role of Mg in enzymatic
424 processes, likely fostering microbial activity and N capture ^{4, 68, 69}. Consistent with the widely assumed
425 importance of initial litter N concentration for litter N dynamics, initial litter N concentration predicted litter
426 N loss but its explanatory power for litter N immobilization was poor. Potentially, this is related to the rather
427 early litter decomposition processes investigated in this study. In a long-term intersite decomposition
428 experiment, Parton et al. ²⁸ found both net N immobilization and release to be controlled predominantly by
429 initial litter N concentration. In our study differences in N loss and ¹⁵N transfer between leaf litter species
430 matched changes in species-specific annual mass loss (Figure S7), suggesting that litter mass loss and N
431 dynamics are more closely linked than generally assumed ^{28, 70}.

432 **4.4 Microarthropod effects on N dynamics**

433 Contrary to our third hypothesis, litter N loss and ¹⁵N transfer into the litter were little affected by access
434 of the litter by microarthropods and this was true for both single litter species and litter mixtures. Generally,
435 this indicates that microarthropods only little affected litter N dynamics during decomposition and suggests
436 that microorganisms and microfauna, such as nematodes and protists, that had access are the main actors
437 responsible for N dynamics in litter of forest ecosystems and litter diversity effects. Litter mixture studies at
438 local and at large spatial scale advanced our knowledge of soil fauna effects on leaf litter decomposition and
439 N dynamics ^{11, 38, 63}. However, previous studies predominantly investigated single litter species and little is
440 known on effects of microarthropods on N dynamics in leaf litter mixtures across large spatial scales
441 hampering extrapolation of previous findings to real-world ecosystems ^{14, 59, 71}. Contrary to single litter species
442 and litter mixtures, results of the present study suggest that N loss and N immobilization in individual leaf
443 litter species in mixtures are modified by access by soil microarthropods, suggesting that effects of
444 microarthropods on litter nutrient dynamics change with plant diversity. Differences in soil fauna community
445 composition and nutritional constraints likely contributed to the different effects of soil microarthropods on
446 litter N dynamics among biomes and litter species, suggesting that microarthropod-mediated changes in litter
447 N dynamics depend on environmental context.

448 **5. Conclusions**

449 Our litter decomposition study across four forest biomes demonstrates that litter mixture strongly affects
450 both N loss and N immobilization, with the effects on N loss being overall positive and the effects on N
451 immobilization being overall negative. However, the relative mixture effects on N dynamics differed among
452 forest biomes, but were little modified by litter species and access by microarthropods. N loss and N
453 immobilization in single litter species and mixtures primarily depended on litter species identity and local
454 environmental context, i.e., forest biome. Two sets of litter characteristics explaining most of the variation in
455 N loss and N translocation, i.e., concentrations of N, Ca and condensed tannins for N loss, and Ca, Mg and
456 condensed tannins for N translocation. Contrary to single litter species and litter mixtures, N loss and N
457 immobilization in individual leaf litter species in mixtures were modified by microarthropod access, suggesting
458 that effects of soil fauna on litter nutrient dynamics change with plant diversity. Increased N emissions due to
459 anthropogenic activities strongly altered forest ecosystem nutrient cycling and ecosystem functioning, but how
460 the additional N altered the dynamics of N in litter and how this feeds back to ecosystem N cycling remains

461 little understood. Results of our study suggest that these effects are likely to vary between pure and mixed
462 forest stands with N immobilization in litter being reduced in mixed stands. To mitigate negative effects of
463 increased N input into forest ecosystems, the drivers of N dynamics in litter and their variations among biomes
464 need closer attention to advance understanding and improve predictions on how increased N deposition
465 impacts nutrient dynamics in pure and mixed forest ecosystems across biomes.

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473 **Authors’ contributions**

474 S.S., and S.H. initiated the idea and designed the study; O.B., and I.T.H. conducted the research and collected
475 the data; S.Z. and C.H. performed statistical analysis; S.Z. and S.S. drafted the manuscript and with
476 contributions from I.T.H., B.M., C.H., and S.H. All authors contributed substantially to the revisions.

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717 **Table 1:** ANOVA table of F- and P-values for the effects of forest biome (FB), litter species identity (SI) and
 718 access of litter by microarthropods (M), and their interactions on (a) litter N loss (% of total initial N, log
 719 transformed) and (b) ^{15}N transfer ($\mu\text{g } ^{15}\text{N g}^{-1}$ dry weight litter, log transformed) in single litter species
 720 treatments. *d.f.* = degrees of freedom, *SS* = Type III sum of squares, *SS%* = percentage of variance explained,
 721 *MS* = mean square. Significant effects are given in bold.

| | <i>Effect</i> | <i>d.f.</i> | <i>SS</i> | <i>SS%</i> | <i>MS</i> | <i>F</i> | <i>P</i> |
|--|-------------------------------|-------------|--------------|--------------|-------------|-------------|-------------------|
| (a) N loss | Forest biome (FB) | 3 | 7.40 | 12.58 | 2.47 | 1.8 | 0.2163 |
| | Species identity (SI) | 3 | 21.72 | 36.90 | 7.24 | 5.8 | 0.0167 |
| | Access by microarthropods (M) | 1 | 0.04 | 0.06 | 0.04 | 0.1 | 0.7409 |
| | FB × SI | 9 | 11.42 | 19.40 | 1.27 | 7.4 | 0.0032 |
| | FB × M | 3 | 0.82 | 1.39 | 0.27 | 1.6 | 0.2578 |
| | SI × M | 3 | 0.59 | 1.00 | 0.20 | 1.1 | 0.3842 |
| | FB × SI × M | 9 | 1.53 | 2.61 | 0.17 | 0.5 | 0.8430 |
| | <i>Residuals</i> | 48 | 15.34 | 26.07 | 0.32 | | |
| (b) ^{15}N transfer | Forest biome (FB) | 3 | 1.28 | 22.20 | 0.43 | 9.8 | 0.0052 |
| | Species identity (SI) | 3 | 3.03 | 52.52 | 1.01 | 30.9 | <0.0001 |
| | Access by microarthropods (M) | 1 | 0.00 | 0.00 | 0.00 | 0.0 | 0.9509 |
| | FB × SI | 9 | 0.30 | 5.15 | 0.03 | 4.0 | 0.0262 |
| | FB × M | 3 | 0.06 | 0.98 | 0.02 | 2.3 | 0.1511 |
| | SI × M | 3 | 0.03 | 0.48 | 0.01 | 1.1 | 0.4070 |
| | FB × SI × M | 9 | 0.07 | 1.30 | 0.01 | 0.4 | 0.9299 |
| | <i>Residuals</i> | 48 | 1.00 | 17.38 | 0.02 | | |

723 **Table 2:** ANOVA table (GLM) of F- and P-values for the effect of forest biome (FB), litter species richness
724 (SR), litter species composition (SC) and access of litter by microarthropods (M), and their interactions on (a)
725 litter N loss (% of initial, log transformed) and (b) ^{15}N transfer ($\mu\text{g } ^{15}\text{N g}^{-1}$ dry weight litter, log transformed)
726 into litter mixtures. *d.f.* = degrees of freedom, *SS* = Type I sum of squares, *MS* = mean square. Significant
727 effects are given in bold.

| | <i>Effect</i> | <i>d.f.</i> | <i>SS</i> | <i>SS%</i> | <i>MS</i> | <i>F</i> | <i>P</i> |
|--|---------------------------------|-------------|-------------|--------------|-------------|-------------|-------------------|
| (a) N loss | Forest biome (FB) | 3 | 0.43 | 0.87 | 0.14 | 0.8 | 0.4745 |
| | Species richness (SR) | 2 | 0.59 | 1.17 | 0.29 | 1.7 | 0.1869 |
| | Species composition (SC) | 8 | 7.62 | 15.22 | 0.95 | 5.5 | <0.0001 |
| | Access by microarthropods (M) | 1 | 0.07 | 0.13 | 0.07 | 0.4 | 0.5372 |
| | FB × SR | 6 | 0.28 | 0.55 | 0.05 | 0.3 | 0.9509 |
| | FB × SC | 24 | 9.26 | 18.50 | 0.39 | 2.2 | 0.0021 |
| | FB × M | 3 | 0.17 | 0.34 | 0.06 | 0.3 | 0.8071 |
| | M × SR | 2 | 0.17 | 0.34 | 0.08 | 0.5 | 0.6145 |
| | M × SC | 8 | 2.41 | 4.82 | 0.30 | 1.7 | 0.0929 |
| | FB × SR × M | 6 | 0.24 | 0.49 | 0.04 | 0.2 | 0.9643 |
| | FB × SC × M | 24 | 6.05 | 12.10 | 0.25 | 1.5 | 0.0913 |
| | <i>Residuals</i> | 132 | 22.76 | 45.48 | 0.17 | | |
| (b) ^{15}N transfer | Forest biome (FB) | 3 | 5.76 | 37.30 | 1.92 | 56.2 | <0.0001 |
| | Species richness (SR) | 2 | 0.01 | 0.05 | <0.01 | 0.1 | 0.8872 |
| | Species composition (SC) | 8 | 2.92 | 18.90 | 0.36 | 10.7 | <0.0001 |
| | Access by microarthropods (M) | 1 | 0.05 | 0.31 | 0.05 | 1.4 | 0.2425 |
| | FB × SR | 6 | 0.11 | 0.68 | 0.02 | 0.5 | 0.7963 |
| | FB × SC | 24 | 0.79 | 5.12 | 0.03 | 1.0 | 0.5172 |
| | FB × M | 3 | 0.21 | 1.37 | 0.07 | 2.1 | 0.1080 |
| | M × SR | 2 | 0.13 | 0.84 | 0.07 | 1.9 | 0.1531 |
| | M × SC | 8 | 0.29 | 1.88 | 0.04 | 1.1 | 0.3951 |
| | FB × SR × M | 6 | 0.05 | 0.35 | 0.01 | 0.3 | 0.9515 |
| | FB × SC × M | 24 | 0.61 | 3.96 | 0.03 | 0.7 | 0.7954 |
| | <i>Residuals</i> | 132 | 4.51 | 29.22 | 0.03 | | |

729 **Table 3:** ANOVA table (GLM) of F- and P-values on the effect of forest biome (FB), litter species richness
730 (SR), litter species composition (SC) and access of litter by microarthropods (M), and their interactions on
731 relative mixture effects on (a) litter N loss (log transformed) and (b) ¹⁵N transfer (log transformed). *d.f.* =
732 degrees of freedom, *SS* = Type I sum of squares, *MS* = mean square. Significant effects are given in bold.

| | <i>Effect</i> | <i>d.f.</i> | <i>SS</i> | <i>MS</i> | <i>F</i> | <i>P</i> |
|------------------------------------|-------------------------------|-------------|---------------|--------------|-------------|-------------------|
| (a) N loss | Forest biome (FB) | 3 | 107.77 | 35.92 | 9.4 | <0.0001 |
| | Species richness (SR) | 2 | 23.23 | 11.62 | 3.0 | 0.0518 |
| | Species composition (SC) | 8 | 42.81 | 5.35 | 1.4 | 0.2041 |
| | Access by microarthropods (M) | 1 | 0.28 | 0.28 | 0.1 | 0.7877 |
| | FB × SR | 6 | 11.25 | 1.88 | 0.5 | 0.8156 |
| | FB × SC | 24 | 68.70 | 2.86 | 0.7 | 0.7951 |
| | FB × M | 3 | 5.66 | 1.89 | 0.5 | 0.6886 |
| | M × SR | 2 | 1.47 | 0.74 | 0.2 | 0.8256 |
| | M × SC | 8 | 32.45 | 4.06 | 1.1 | 0.3966 |
| | FB × SR × M | 6 | 4.02 | 0.67 | 0.2 | 0.9833 |
| | FB × SC × M | 24 | 39.02 | 1.63 | 0.4 | 0.9916 |
| | <i>Residuals</i> | 132 | 506.23 | 3.84 | | |
| (b) ¹⁵N transfer | Forest biome (FB) | 3 | 42.16 | 14.05 | 11.4 | <0.0001 |
| | Species richness (SR) | 2 | 5.32 | 2.66 | 2.2 | 0.1193 |
| | Species composition (SC) | 8 | 10.93 | 1.37 | 1.1 | 0.3611 |
| | Access by microarthropods (M) | 1 | 0.11 | 0.11 | 0.1 | 0.7688 |
| | FB × SR | 6 | 2.72 | 0.45 | 0.4 | 0.8978 |
| | FB × SC | 24 | 26.76 | 1.12 | 0.9 | 0.5948 |
| | FB × M | 3 | 13.75 | 4.58 | 3.7 | 0.0131 |
| | M × SR | 2 | 1.07 | 0.54 | 0.4 | 0.6480 |
| | M × SC | 8 | 6.05 | 0.76 | 0.6 | 0.7653 |
| | FB × SR × M | 6 | 3.64 | 0.61 | 0.5 | 0.8130 |
| | FB × SC × M | 24 | 32.91 | 1.37 | 1.1 | 0.3385 |
| | <i>Residuals</i> | 132 | 162.62 | 1.23 | | |

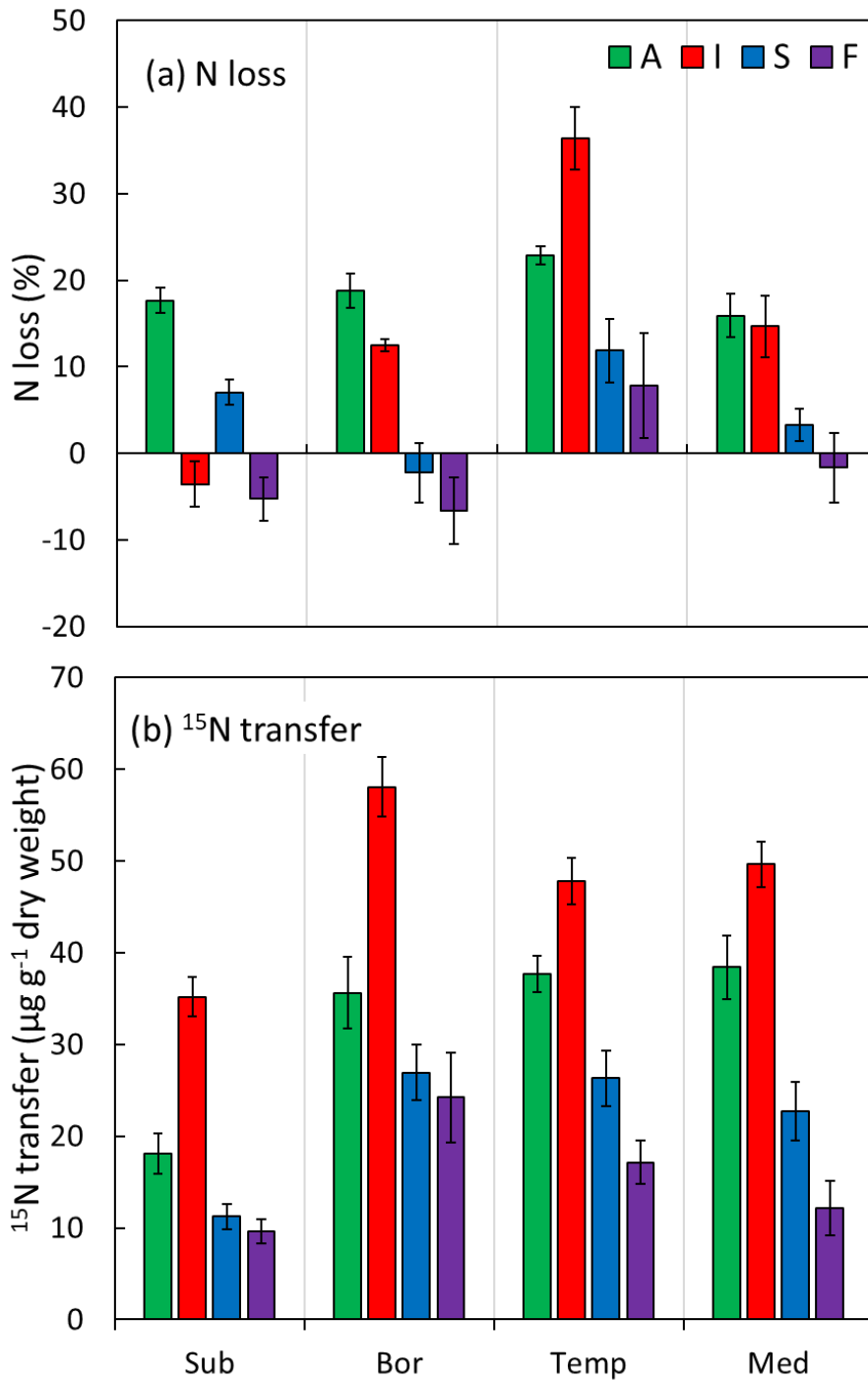
734 **Table 4:** ANOVA table (GLM) of F- and P-values for the effect of forest biome (FB), litter species identity
735 (SI), litter species richness (SR), litter species composition (SC) and access of litter by microarthropods (M)
736 and their interactions on mixture effects on (a) N loss and (b) ¹⁵N transfer into individual litter species within
737 mixtures. *d.f.* = degrees of freedom, *SS* = Type I sum of squares, *MS* = mean square. Significant effects are
738 given in bold.

| | <i>Effect</i> | <i>d.f.</i> | <i>SS</i> | <i>MS</i> | <i>F</i> | <i>P</i> |
|------------------------------|---------------------------------|-------------|---------------|--------------|-------------|-------------------|
| (a) N loss | Forest biome (FB) | 3 | 123.71 | 41.24 | 12.6 | <0.0001 |
| | Species identity (SI) | 3 | 272.70 | 90.90 | 27.8 | <0.0001 |
| | Species richness (SR) | 2 | 4.12 | 2.06 | 0.6 | 0.5329 |
| | Species composition (SC) | 8 | 28.73 | 3.59 | 1.1 | 0.3633 |
| | Access by microarthropods (M) | 1 | 3.14 | 3.14 | 1.0 | 0.3277 |
| | FB × SI | 9 | 116.95 | 12.99 | 4.0 | 0.0001 |
| | FB × SR | 6 | 23.74 | 3.96 | 1.2 | 0.3001 |
| | FB × SC | 24 | 50.29 | 2.10 | 0.6 | 0.9059 |
| | FB × M | 3 | 6.76 | 2.25 | 0.7 | 0.5593 |
| | SI × SR | 6 | 0.82 | 0.14 | 0.0 | 0.9997 |
| | SI × SC | 8 | 16.05 | 2.01 | 0.6 | 0.7670 |
| | SI × M | 3 | 0.52 | 0.17 | 0.1 | 0.9840 |
| | M × SR | 2 | 4.93 | 2.46 | 0.8 | 0.4714 |
| | M × SC | 8 | 31.24 | 3.91 | 1.2 | 0.3008 |
| | FB × SI × M | 9 | 71.50 | 7.94 | 2.4 | 0.0105 |
| <i>Residuals</i> | 464 | 1517.90 | 3.27 | | | |
| (b) ¹⁵ N transfer | Forest biome (FB) | 3 | 64.73 | 21.58 | 13.2 | <0.0001 |
| | Species identity (SI) | 3 | 84.74 | 28.25 | 17.3 | <0.0001 |
| | Species richness (SR) | 2 | 6.29 | 3.14 | 1.9 | 0.1473 |
| | Species composition (SC) | 8 | 30.93 | 3.87 | 2.4 | 0.0168 |
| | Access by microarthropods (M) | 1 | 0.02 | 0.02 | 0.0 | 0.9174 |
| | FB × SI | 9 | 56.25 | 6.25 | 3.8 | 0.0001 |
| | FB × SR | 6 | 12.29 | 2.05 | 1.3 | 0.2782 |
| | FB × SC | 24 | 31.71 | 1.32 | 0.8 | 0.7280 |
| | FB × M | 3 | 15.70 | 5.23 | 3.2 | 0.0232 |
| | SI × SR | 6 | 2.80 | 0.47 | 0.3 | 0.9437 |
| | SI × SC | 8 | 16.47 | 2.06 | 1.3 | 0.2631 |
| | SI × M | 3 | 6.72 | 2.24 | 1.4 | 0.2516 |
| | M × SR | 2 | 10.72 | 5.36 | 3.3 | 0.0386 |
| | M × SC | 8 | 6.31 | 0.79 | 0.5 | 0.8689 |
| | FB × SI × M | 9 | 56.14 | 6.24 | 3.8 | 0.0001 |
| <i>Residuals</i> | 464 | 758.75 | 1.64 | | | |

740 **Table 5:** Results of partial least square (PLS) regressions for the effects of fixed subsets of litter traits on (a)
741 litter N loss (% of initial, log transformed) and (b) ^{15}N transfer ($\mu\text{g } ^{15}\text{N g}^{-1}$ dry weight litter, log transformed)
742 in single litter species and litter mixtures across four forest biomes and separately in the subarctic (Sub), boreal
743 (Bor), temperate (Temp) and Mediterranean biome (Med). N = nitrogen, Ca = calcium, Mg = magnesium, Tan
744 = condensed tannins. *VIP* = importance of the predictors in the model, R^2X = variance of predictors in the
745 model, R^2Y = variance of the dependent variable explained by the model.

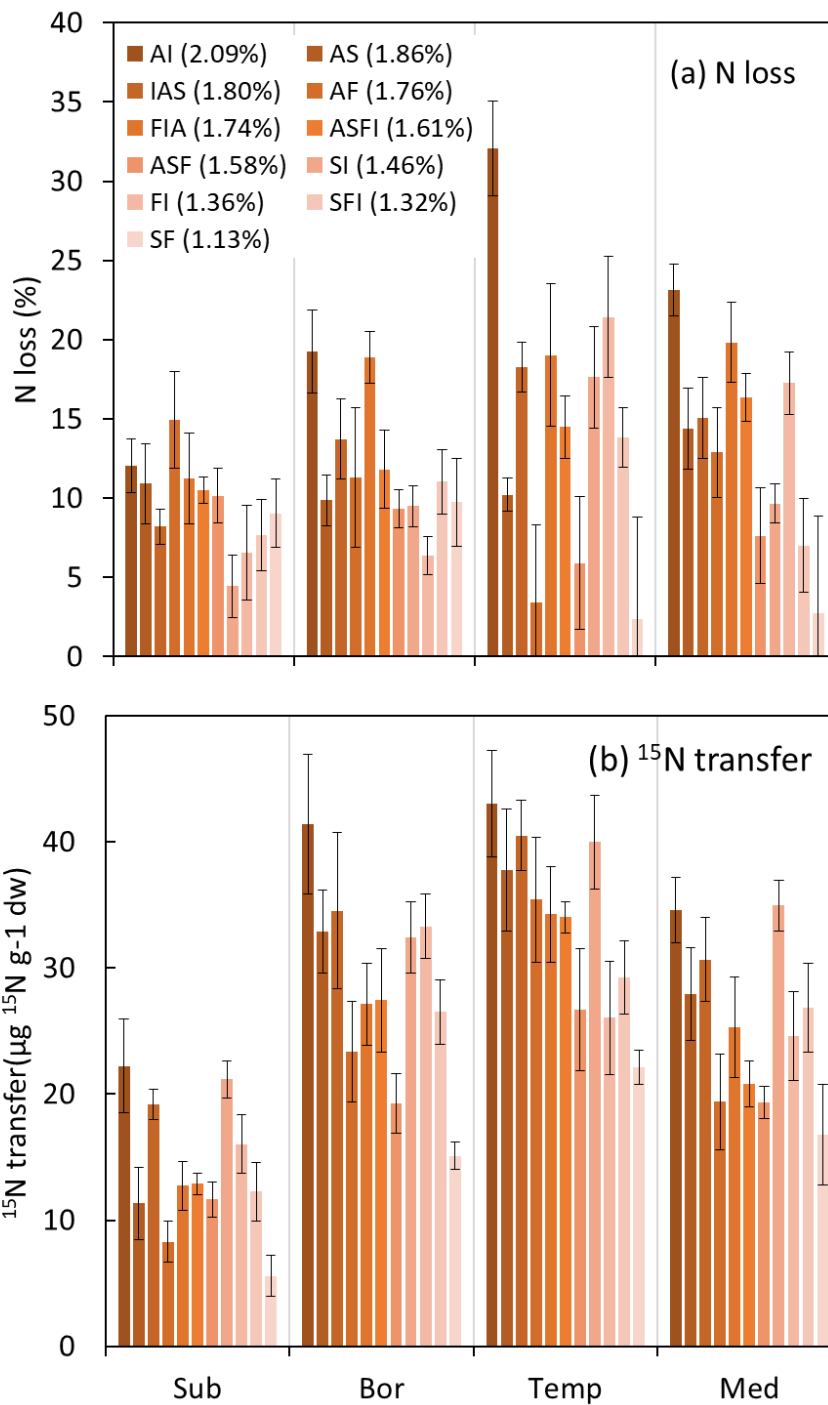
| | Gradient | | Sub | | Bor | | Temp | | Med | | |
|------------------------------|------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------|
| | Single | Mixture | Single | Mixture | Single | Mixture | Single | Mixture | Single | Mixture | |
| | <i>Predictor</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | <i>VIP</i> | |
| (a)N loss | N | 1.05 | 0.93 | 1.37 | 1.66 | 0.97 | 0.87 | 0.82 | 0.59 | 0.94 | 0.94 |
| | Ca | 1.04 | 0.95 | 0.99 | 0.04 | 0.98 | 1.10 | 1.14 | 1.16 | 1.04 | 0.90 |
| | TAN | 0.90 | 1.11 | 0.39 | 0.49 | 1.05 | 1.02 | 1.01 | 1.15 | 1.02 | 1.14 |
| | R^2X | 0.87 | 0.87 | 0.86 | 0.79 | 0.87 | 0.87 | 0.86 | 0.86 | 0.87 | 0.87 |
| | R^2Y | 0.33 | 0.13 | 0.21 | 0.05 | 0.70 | 0.10 | 0.45 | 0.26 | 0.50 | 0.25 |
| (b) ^{15}N transfer | Ca | 1.01 | 0.98 | 0.95 | 0.91 | 0.95 | 1.01 | 1.05 | 1.15 | 1.06 | 0.99 |
| | Mg | 1.01 | 1.02 | 1.09 | 1.11 | 1.08 | 1.01 | 0.95 | 0.82 | 0.93 | 0.99 |
| | TAN | 0.98 | 1.00 | 0.96 | 0.97 | 0.97 | 0.98 | 1.00 | 1.00 | 1.00 | 1.03 |
| | R^2X | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 |
| | R^2Y | 0.52 | 0.17 | 0.65 | 0.25 | 0.58 | 0.47 | 0.77 | 0.33 | 0.71 | 0.35 |

746



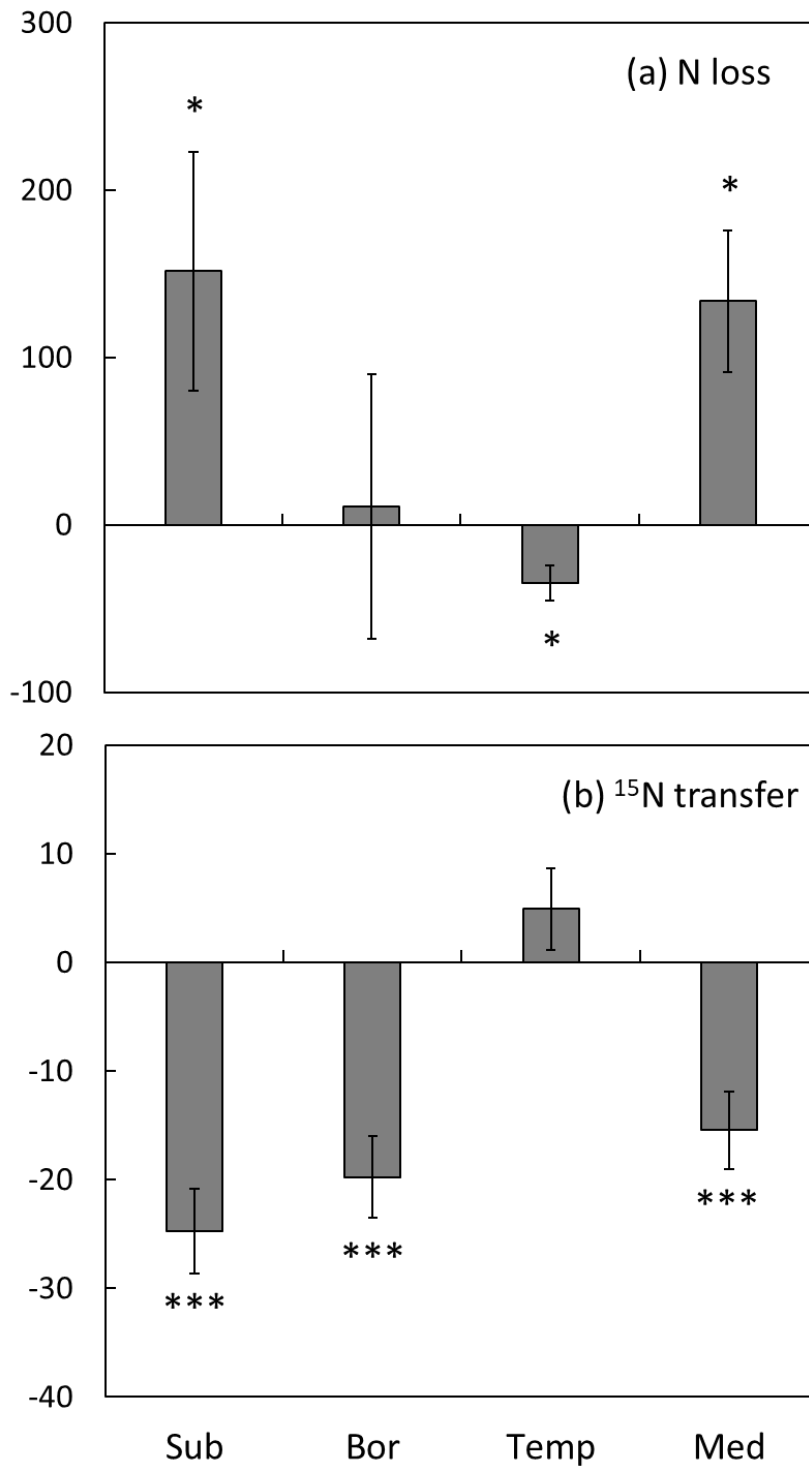
748

749 **Figure 1** Average litter (a) N loss (%) and (b) ¹⁵N transfer (µg ¹⁵N g⁻¹ dry weight litter) in leaf litter of *Alnus*
 750 *glutinosa* (A), *Ilex aquifolium* (I), *Salix cinerea* (S) and *Fagus sylvatica* (F) incubated as single litter species
 751 in the subarctic (Sub), boreal (Bor), temperate (Temp) and Mediterranean forest (Med) (means ± SE). Species
 752 are plotted in the order of decreasing initial litter N concentration. Data are pooled across microarthropod
 753 treatments. Means of six replicates in the subarctic and Mediterranean forest, and four in the temperate and
 754 boreal forest (see Material and Methods).



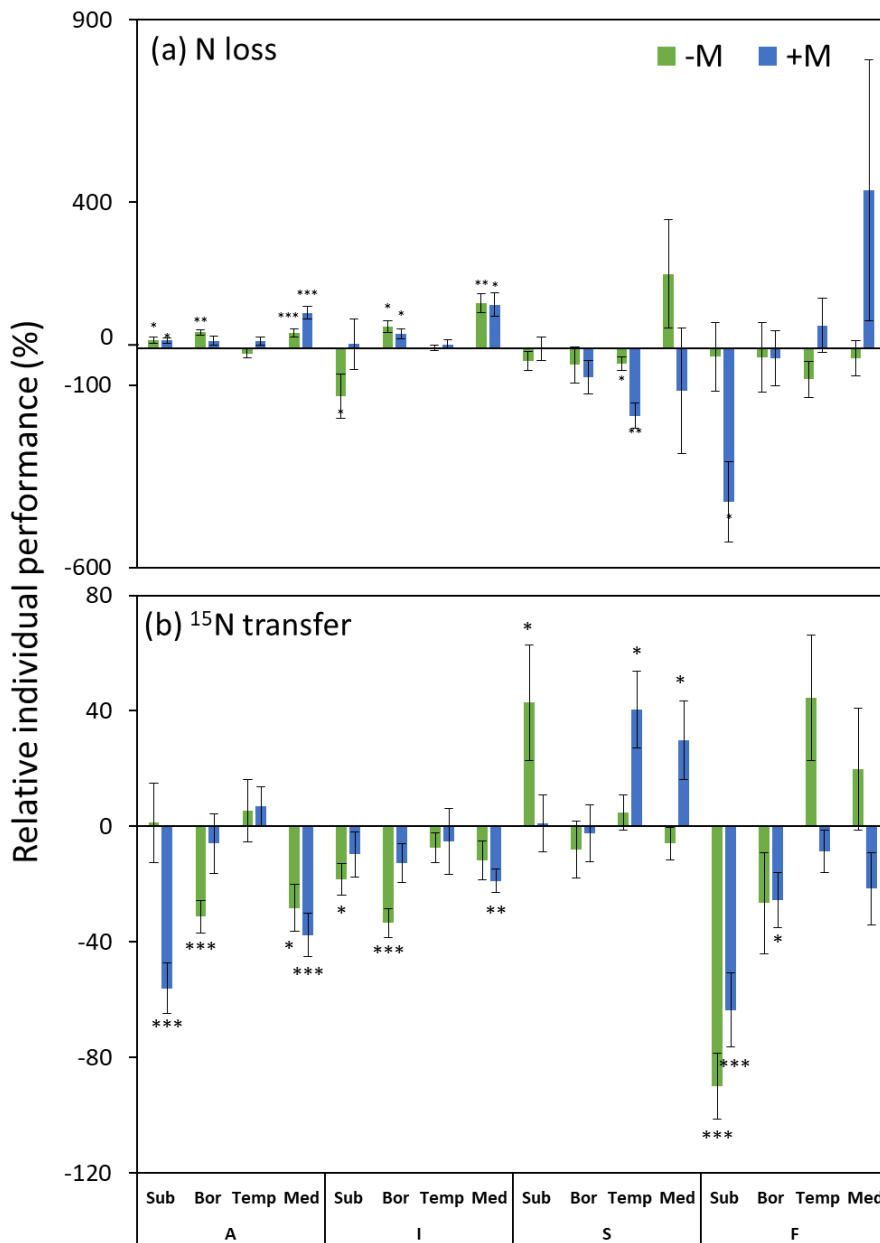
755

756 **Figure 2** Average litter (a) N loss (%) and (b) ¹⁵N transfer (µg ¹⁵N g⁻¹ dry weight litter) in *Alnus glutinosa* (A),
 757 *Ilex aquifolium* (I), *Salix cinerea* (S) and *Fagus sylvatica* (F) in mixtures in the subarctic (Sub), boreal (Bor),
 758 temperate (Temp) and Mediterranean forest (Med) (means ± SE). Data are pooled across microarthropod
 759 treatments. Mixtures are ordered by decreasing initial average N concentrations given in brackets (decreasing
 760 color intensity). Means of six replicates in the subarctic and Mediterranean forest, and four in the temperate
 761 and boreal forest (see Material and Methods).



762

763 **Figure 3** Relative mixture effects on leaf litter (a) N loss and (b) ¹⁵N transfer across litter mixtures and
 764 microarthropod treatments (means ± SE). Sub: Subarctic forest, Bor: boreal forest, Temp: temperate forest and
 765 Med: Mediterranean forest. Means of 66 replicates in the subarctic and Mediterranean forest, and 44 in the
 766 temperate and boreal forest (see Material and Methods). Asterisks indicate significant deviations from zero
 767 (Student's t tests; * < 0.05, *** < 0.0001).



768

769 **Figure 4** Relative (a) N loss and (b) ¹⁵N transfer into individual litter species in mixtures without (-M) or with
 770 (+M) access by microarthropods (means ± SE). Means of 21 replicates for each species in the subarctic and
 771 Mediterranean forest, and 14 in the temperate and boreal forest (see Material and Methods). See Figure 1 for
 772 abbreviations of litter species and biomes. Asterisks indicate significant deviations from zero (Student's t tests;
 773 * < 0.05, ** < 0.001, *** < 0.0001).