Consequences of biodiversity loss for litter decomposition across biomes

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Abstract: The decomposition of dead organic matter is a major determinant of carbon and nutrient cycling in ecosystems, and of carbon fluxes between the biosphere and the atmosphere. Decomposition is driven by a vast diversity of organisms that are structured in complex food webs. Identifying the mechanisms underlying the effects of biodiversity on decomposition is critical given the rapid loss of species worldwide and the effects of this loss on human well-being. Yet despite comprehensive syntheses of studies on how biodiversity affects litter decomposition, key questions remain, including when, where and how biodiversity has a role and whether general patterns and mechanisms occur across ecosystems and different functional types of organism. Here, in field experiments across five terrestrial and aquatic locations, ranging from the subarctic to the tropics, we show that reducing the functional diversity of decomposer organisms and plant litter types slowed the cycling of litter carbon and nitrogen. Moreover, we found evidence of nitrogen transfer from the litter of nitrogen-fixing plants to that of rapidly decomposing plants, but not between other plant functional types, highlighting that specific interactions in litter mixtures control carbon and nitrogen cycling during decomposition. The emergence of this general mechanism and the coherence of patterns across contrasting terrestrial and aquatic ecosystems suggest that biodiversity loss has consistent consequences for litter decomposition and the cycling of major elements on broad spatial scales.

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Consequences of biodiversity loss for litter decomposition across biomes

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[‡] In fond memory of our late colleague and friend

Carbon (C) and nitrogen (N) dynamics during litter decomposition are fundamentally important in controlling global biogeochemical cycles, nutrient availability, and primary productivity[1,2,3]. Decomposition is driven by a vast diversity of life structured in complex food webs[2,4]. Identifying the mechanisms underlying biodiversity effects on decomposition is critical[4-6] given the rapid loss of species worldwide and its impact on humanity[7-9]. Yet, despite comprehensive syntheses[4-6,10], key questions remain, including when, where and how biodiversity plays a role, and whether general patterns and mechanisms occur across different functional types of organisms and ecosystems[4,9-12]. Here we find in concerted field experiments across five terrestrial and aquatic locations ranging from the subarctic to the tropics, that the loss of consumer and litter functional diversity slowed the cycling of litter C and N. Moreover, evidence for N transfer from litter of N-fixing to rapidly decomposing plants, but not between other plant functional types, highlights that specific interactions in litter mixtures control C and N cycling during decomposition. Emergence of this general mechanism and the coherence of patterns across contrasting terrestrial and aquatic ecosystems suggest consistent consequences of biodiversity loss on litter decomposition and the cycling of major elements at broad spatial scales.

Main text

Biological diversity that directly influences litter decomposition exists at multiple trophic levels[4]. This diversity includes plants producing litter mixtures of varying quality, microbial decomposers, and invertebrate consumers of varying body size, which selectively exploit the heterogeneous resources provided by litter mixtures[4,13]. Efforts to derive generalities about biodiversity effects on litter decomposition have been elusive, since both pioneering work[14] and recent syntheses have highlighted contrasting effects of litter species richness on

decomposition[4-6,15,16]. In part, this variation appears to be due to site-specific conditions, including contrasts between aquatic and terrestrial ecosystems as well as geographic settings. Further differences may arise from variation in experimental protocols, selected plant species, and the types of decomposers included in a given experiment. Such methodological discrepancies have complicated syntheses across studies, hindering the emergence of common patterns and mechanisms.

Here we report on the results from the first concerted biodiversity experiments on decomposition by manipulating diversity across trophic levels and distinct biomes in both forest floor and stream habitats (Extended Data Table 1). We hypothesised that functional diversity of decomposers (variation in body size) and leaf litter (variation in litter quality) promote C and N cycling across contrasting locations (subarctic to tropical) and ecosystem types (terrestrial *vs.* aquatic). Body size encapsulates numerous species traits relevant for ecosystem functioning, and extinction scenarios project preferential loss of the larger species from biological communities[17,18]. Similarly, plant functional types reflect differences in leaf quality traits determining litter decomposition independent of geographical location[19]. Plant functional types are defined here in terms of plant C allocation strategy (deciduous *versus* evergreen), N acquisition strategy (N-fixing *versus* non N-fixing), and litter recalcitrance (rapidly *versus* slowly decomposing) (Extended Data Table 2).

Litter mixing resulted in accelerated C and N dynamics, as indicated by overall positive net diversity effects of C and N loss from litter mixtures (Fig. 1 for C loss, Extended Data Fig. 1 for N loss, $P < 0.05$ for C and N loss). However, N loss was only 0.6±0.3% (mean±SE), and C loss 4.2±0.9% greater across all mixtures, indicating rather modest increases in the cycling of both elements in litter mixtures. The net litter diversity effect on C loss was stronger in terrestrial than in aquatic ecosystems ($P < 0.001$, Fig. 1, Extended Data Table 3), supporting theoretical predictions[4] but contrasting a meta-analysis in which diversity effects on decomposition were

significant only for streams[6]. Sorting litter mixtures to species at the end of the experiments enabled us to explore potential reasons for this discrepancy by partitioning net diversity effects into complementarity effects (i.e. effects resulting from synergistic or antagonistic interactions), and effects arising when a dominant species in mixtures accelerates (or slows) process rates above (or below) those of the constituent species decomposing singly (i.e. selection effects)[20]. The observed net diversity effect was clearly driven by complementarity effects (Fig. 1). It was similarly strong as the net effect for C loss (+4.9±0.9%), and even stronger for N loss (+1.8±0.3%), whereas selection effects were weak (-1.0±0.5% for C and -0.6±0.4% for N). Characteristics of the forest floor habitat that may favour complementarity effects, include strong fluctuations in temperature and humidity, and a homogenous litter cover[4]. Conversely, the observed negative complementarity effects in subarctic and tropical streams could reflect low densities and taxon richness of litter consumers (Extended Data Table 4), and thus limited potential for complementary resource use[21].

Our experiments also show that completeness of the decomposer community, which is rarely considered in large-scale studies, is important for C and N dynamics (Fig. 2, Table 1, Extended Data Table 5). Presence of medium-sized invertebrates in the decomposer community increased the average C and N loss across all sites by 2.1±0.8% and 2.0±1.0%, respectively. The complete decomposer community accelerated the average C loss by 10.6±1.0% and the average N loss by 11.1±1.2% (Fig. 2). This effect was consistently positive across all but the Mediterranean terrestrial site. Thus, large fauna clearly has a major impact on decomposition (Table 1) , as reported previously[22-24], but in line with former studies, its importance varies among locations in aquatic[22] and terrestrial ecosystems[23,24]. In our study, the strong effects of the complete decomposer community at the temperate and tropical locations correspond with high relative abundances of millipedes and termites at the temperate and tropical sites, respectively (Extended Data Table 6). Similarly, the large effect of the complete decomposer community at

the temperate aquatic site matches the high abundance of a particularly efficient amphipod detritivore (Extended Data Table 4). Our data clearly indicate that loss of the large-bodied fauna was the most critical for decomposition. Those animals also tend to face the greatest extinction risk[17].

Litter mixing and completeness of the decomposer community interactively affected C and N loss, although this interaction explains less of the variance than the main effects (Table 1). Carbon loss and, even more so, N loss increased in the presence of particular plant functional types and with increasing completeness of the decomposer community (Table 1, Extended Data Table 7). Although the type of decomposer community was not significant in explaining the net diversity effect on C ($P = 0.48$) or N loss ($P = 0.09$, Extended Data Table 3), it emerged as a significant factor in explaining the selection effect for both C ($P < 0.05$) and N loss ($P < 0.01$). Additionally, the interaction between the rapidly decomposing litter type and the decomposer community was significant in explaining the selection and overall net diversity effect on C and N loss ($P < 0.05$), suggesting that large decomposers are particularly important drivers of C and N loss from litter mixtures containing rapidly decomposing litter. Food preference behaviour could be important to account for this result, as previously implied for terrestrial[25] and aquatic ecosystems[26].

A key result of our large-scale study is that litter diversity effects on C and N dynamics could be largely explained by the presence of particular functional plant types in litter mixtures, supporting the idea that the range and relative abundance of plant traits in ecosystems underlie the effects of species richness on ecosystem processes[27,28]. Effects of the presence of, or interactions among, litter of particular plant functional types were consistent across locations at both terrestrial and aquatic sites, together accounting for about 10% of the total variance (Table 1, $P < 0.05$; Extended Data Table 7). Beyond the presence or absence of particular functional types, we found no significant effect of litter plant functional type richness on C loss ($F_{1,1739} =$

0.01, $P = 0.93$), although a positive effect was observed on N loss ($F_{1,1739} = 63.7$, $P < 0.001$, Table 1). The latter was strongest when the most complete decomposer communities had access to the litter (Litter richness × Decomposer community interaction; $F_{2,1739} = 3.45$, $P < 0.05$). These results indicate that partitioning diversity effects into separate contributions of the presence or absence of particular litter functional types and their interactions can help move interpretations of biodiversity-ecosystem functioning experiments beyond the current dichotomy between broad generalisations and claims of idiosyncratic compositional effects[5,14,15].

An intriguing finding in this context is that the strongest positive interaction emerged between two particular litter functional types, N-fixing and rapidly decomposing deciduous plants (Extended Data Table 7). When both occurred together in litter mixtures, the average C loss was 13.5% greater and the N loss even 32.5% greater than the average of all litter combinations. This general pattern holds across terrestrial and aquatic ecosystems from the subarctic to the tropics. Moreover, relative to the total amount of N in litter initially, less N remained in litter of the N-fixing plants when rapidly decomposing litter was present, which in turn contained more N than when it decomposed alone (Fig. 3, Extended Data Table 8). On average across all sites, litter of N-fixing plants lost 20.6% of their initial amount of N when decomposing alone as compared to 25.0% when decomposing in the presence of litter from rapidly decomposing plants. In contrast, litter of rapidly decomposing plants lost 18.1% of their amount of N when decomposing alone compared to 13.4% when litter from N-fixing plants was present. This striking pattern across locations and ecosystems suggests, for the first time from field data, that N can be transferred between litter types. A plausible mechanism for this effect is that fungal decomposers tapped the nutrient reservoir of the N-fixing plant litter to boost C use and fungal growth in the N-deficient litter that provided high-quality C[29] (see Extended Discussion on N transfer). The average net differences in N fluxes between single-species litter and litter mixtures of these two plant functional types account for approximately 0.25 g of N per

square meter of ground area, representing up to a tenth of the total annual N input from leaf litter fall. Thus, although our reported biodiversity effects, in line with recent syntheses[9-11], are smaller than noted for other ecosystem processes such as plant biomass production, these changes in N fluxes can have important ecosystem consequences. Even slight differences in N dynamics in litter mixtures compared to the respective single litter species can substantially change N supply to primary producers over large spatial and temporal scales[30].

The implications of our results are that changes in C and N cycling are largely predictable across vastly different latitudes in both terrestrial and aquatic ecosystems from relatively simple plant traits and structural characteristics of decomposer communities. Mechanisms resulting from specific interactions between biodiversity components we describe, are essential to providing robust projections of ecosystem responses to biodiversity loss. With the consistent patterns and mechanisms of biodiversity effects demonstrated here, such projections now appear to be within reach.

Methods summary

Field experiments followed an identical protocol at ten sites, encompassing both aquatic (forest stream) and terrestrial (forest floor) ecosystems at five locations across a latitudinal gradient spanning from the subarctic to the tropics with intermediate locations in boreal, temperate and Mediterranean climates (Extended Data Table 1). Leaf litter from native tree or shrub species representing four common functional types (evergreen, deciduous with slowly decomposing litter, deciduous with rapidly decomposing litter, N-fixing) that naturally occur across all locations (18 species in total; Extended Data Table 2) was exposed to decomposers in a total of 2250 experimental microcosms set up in the field with all possible location-specific single-species and multi-species combinations. We used a randomized block design with 5 blocks per site. Each block contained 1 replicate of 15 combinations of litter types (i.e. all possible

combinations of four litter species) × 3 microcosm mesh sizes (= 45 microcosms per block). Three different mesh sizes used to construct the field microcosms allowed us to establish three different, increasingly complete (small, medium-sized and complete), decomposer communities in the microcosms. Small-sized decomposer communities included microorganisms and fauna passing 50-µm and 250-µm mesh screens in terrestrial and aquatic ecosystems, respectively. The medium-sized decomposer communities also comprised invertebrates passing through 1-mm mesh screens, whereas the full decomposer communities included all animals that passed through 5-mm mesh screens. Litter mass loss was allowed to proceed to the same defined decomposition stage (40–50% of mass remaining of the least recalcitrant litter type at each location; Extended Data Table 9) to ensure comparisons for C and N loss as well as diversity effects at a similar decomposition stage among sites using analysis of variance models (see Extended Methods section).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author contributions
All authors contributed to experimental design, data acquisition and revision of the final manuscript. Statistical analyses were performed by ITH, BS, JvR, and BGM, and the manuscript was written by ITH, SH, and MOG.

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Table 1 Relative contributions of variance associated with diversity and sites expressed in percent sums of squares (% SS) to explain C and N loss in a large-scale leaf litter decomposition experiment. Grey panels highlight main factors. P<0.001***. See Extended Data Table 5 for details.

FACTOR		C LOSS			N LOSS		
Variation associated with diversity	**DF**	**%SS**	**F**	**P**	**%SS**	**F**	**P**
Litter community							
Litter richness*	1	0	0	0.93	1.0	63.7	***
Remaining variation associated with functional type composition†	13	10.1	66.0	***	8.9	44.4	***
Decomposer community (Small, medium-sized or complete decomposer community)	2	5.8	247	***	4.4	142	***
Litter community × Decomposer community	28	0.8	2.45	***	1.3	3.1	***
Variation associated with sites							
Location (Tropical, Mediterranean, temperate, boreal or subarctic)	4	12.1	52.8	***	16.9	66.5	***
Ecosystem (Aquatic stream or terrestrial forest floor)	1	6.4	113	***	1.4	22.3	***
Location × Ecosystem	4	6.3	27.7	***	8.0	31.6	***
Block (within Location × Ecosystem)	40	2.3	4.87	***	2.5	4.13	***
Variation associated with diversity or sites							
Decomposer community × Location	8	8.6	92.1	***	7.5	61.3	***
Remaining variance	388	27.1	5.95	***	21.4	3.60	***
Total variance explained by the model	489	79.5	13.9	***	73.3	9.76	***
Residuals	1739	20.4			26.7		
Total	2228	100.0			100.0		

*Plant species were selected to represent the same four functional types (FT) at each location (N-fixing, evergreen, rapidly or slowly decomposing deciduous trees/shrubs). Linear functional type richness was fitted prior to litter FT compositions.

†An alternative model omitting richness and testing in detail litter FTcompositions in a full factorial analysis with contrasts for FT presence/absence and interactions is presented in Extended Data Table 7. That model highlights the importance of the interaction between litter of N-fixing and rapidly decomposing functional types hinting at a N-transfer mechanism.

Figure legends

Figure 1 Net diversity, complementarity and selection effects of all plant litter mixtures for C loss. Net diversity effects are the deviations from the expected mean based on C loss measured from single litter species. Blue and brown circles show mean effects (±SE) in forest streams and on forest floors, respectively, in subarctic (SUB), boreal (BOR), temperate (TEM), Mediterranean (MED) and tropical (TRO) climates. Each symbol shows the mean effect per ecosystem type calculated across the three types of decomposer communities (n=165 litter mixtures per location and ecosystem type, see Extended Data Table 3 for statistics).

Figure 2 Effect of decomposer community completeness on litter C loss (left panel) and N loss (right panel) loss. Top panels show effects of medium-sized decomposers (percent difference compared to the smallest mesh size) and bottom panels show effects of the complete decomposer community (percent difference compared to the smallest mesh size). Blue and brown bars show mean effects (±SE) in streams and on forest floors, respectively, in subarctic (SUB), boreal (BOR), temperate (TEM), Mediterranean (MED) and tropical (TRO) location (n=45 litter treatments per location per ecosystem type, see Table 1 for statistics).

Figure 3 Relative change in the total amount of litter N. The net difference between two-species mixtures and monocultures for either the N-fixing or the rapidly decomposing plant is shown (mean±SE, n=15, see Extended Data Table 8 for statistics), calculated as $(N_{i,m}–N_{f,m})/N_{i,m}$-$(N_{i,a}–N_{f,a})/N_{f,a})$, where $N_{i,m}$ and $N_{i,a}$ are the initial (i) and $N_{f,m}$, and $N_{f,a}$ are the final (f) amounts of N of a particular litter type in a mixture (m) or alone (a). Litter of N-fixing plants (left panel) and rapidly decomposing litter types (right panel) decomposing in terrestrial (brown bars) and aquatic (blue bars) ecosystems at five locations.

Methods

Experimental design

Our field experiments followed an identical protocol at a total of ten sites, representing either an aquatic ecosystem (forest stream) or a terrestrial ecosystem (forest floor). Five locations were selected across a broad latitudinal gradient spanning from the subarctic to the tropics, with intermediate locations in boreal, temperate and Mediterranean climates (Extended Data Table 1). Across all five locations and in both stream and forest ecosystems, experiments consisted of a randomized block design in which litter of four common native plants (corresponding to the functional types shown in Extended Data Table 2) and eleven mixtures of these litter types (corresponding to all possible litter combinations within a location) were enclosed in nylon mesh screens and placed in the field in five blocks (n = 5 locations × 2 ecosystem types × 15 litter combinations × 3 mesh sizes × 5 blocks = 2250 microcosms). The four functional plant types represent distinct plant C allocation strategies (deciduous *versus* evergreen trees), N acquisition strategies (N-fixer *versus* non N-fixer), and litter recalcitrance of deciduous non N-fixers (rapidly decomposing *versus* slowly decomposing).

Three different mesh sizes used to construct the microcosms enabled us to distinguish three different, increasingly complete decomposer communities (small, medium-sized and complete) establishing on the decomposing litter. Small decomposers included microorganisms and small-sized fauna passing 50-µm and 250-µm mesh screens in terrestrial and aquatic systems, respectively. The medium-sized decomposer communities comprised invertebrates that passed through 1-mm mesh screens, whereas complete decomposer communities included all decomposers passing through 5-mm mesh screens. Litter mass loss was allowed to proceed to the same defined decomposition stage (40–50% of remaining litter mass of the least recalcitrant litter type at each site; Extended Data Table 9) to ensure meaningful comparisons of C and N loss

among all sites. At all ten sites, extra microcosms containing the fastest decomposing litter type served as a benchmark indicator for decomposition rates.

Site characterisation

The five stream locations were characterised in terms of geomorphological, physical and chemical features (Extended Data Table 1). Water samples were collected for chemical analyses when the experiments were established. Samples for inorganic N and P determination were filtered over cellulose acetate 0.45-µm pore-size membrane filters and transported to the laboratory in a cooler, where they were frozen for later analysis at Eawag, Switzerland.

At the five forest sites (Extended Data Table 1), leaf area index was measured at breast height on a uniformly cloud-covered day when the forest canopy was fully developed (LAI 2000 (subarctic) or LAI 2200 (Mediterranean and tropical), Li-Cor, Lincoln, USA, or Sun Scan Canopy Analysis System (temperate), Delta T Devices Ltd., Burwell Cambridge, UK). To characterise the soil at each of the terrestrial sites, three samples from each experimental block were taken with a soil corer (∅ 5 cm, 10 cm height), pooled, stored in plastic bags at 4°C, and later sent cooled to the University of Göttingen, Germany. Sieved soil samples (< 2 mm) were analysed for pH (2 g of soil in 20 ml 0.01 M $CaCl_2$) and C and N concentration (NA 1500, Carlo Erba elemental analyzer, Milan, Italy). Soil microbial biomass was estimated using the substrate-induced respiration (SIR) method. The microbial respiratory response was measured in an electrolytic O_2-microcompensation apparatus at 22°C. These measurements were made hourly for 24 h. Microbial biomass was measured after the addition of glucose as substrate to saturate the catabolic activity of the microorganisms. The maximum initial respiratory response (MIRR; µl O_2 g^{-1} dry mass h^{-1}) was calculated as the mean of the lowest three readings within the first 10 h and microbial biomass was calculated as: $C_{mic} = 38 \times MIRR$ (µg C_{mic} g^{-1} soil dry mass).

Data loggers were installed in some microcosms at all 10 sites to record temperature at bihourly intervals. Measurements were taken in the same litter treatment for all three mesh sizes in three of the five experimental blocks.

Leaf litter collection

A total of 20 litter types was collected at the five locations of our coordinated experiment. This litter corresponded to the same four functional types per location introduced above: N-fixing plants, rapidly decomposing deciduous plants, slowly decomposing deciduous plants, and broad-leaved evergreens (Extended Data Table 2). Litter from these four functional types vary in a number of quality traits (Extended Data Table 2)[31]. The selected species were common native trees or, in two cases, native woody shrubs (*Vaccinium vitis-idaea* and *Rhododendron tomentosum*) occurring at each location. The litter was collected during location-specific leaf senescence either by hand (*V. vitis-idaea* and *R. tomentosum*) or by means of litter traps. An exception was litter of the temperate evergreen species, *Ilex aquifolium*, which was obtained by cutting branches in the field and simulating senescence in the laboratory for three weeks. Leaves with signs of herbivory or disease were discarded. Litter from multiple individual trees or shrubs of each species were pooled and dried at 40°C.

Leaf litter field incubations

Stream experiments were conducted by exposing litter batches of 5 g in tetrahedral mesh microcosms (17 cm × 25 cm) made of one of three mesh sizes (250 μm, 1 mm or 5 mm) to provide access to decomposer communities differing in body size. The microcosms were randomly attached (about 40 cm distance) to five 20-m metal chains, each in a separate riffle 20 m or farther apart from each other (experimental blocks). The chains were fixed in the stream with rebars in fairly homogeneous sand-gravel stream sections where leaves accumulated

naturally. All microcosms were submerged at depths sufficient to ensure that they were not exposed to air when water levels dropped. Care was taken to expose the litter to constant flow conditions, avoiding deep depositional areas (i.e. pools, backwaters) with slow or no flow or rocky riffles with broken flow.

Terrestrial experiments on the forest floor were conducted by incubating 8 g of the location-specific litter (4 g only in the subarctic because of limited litter availability for some species) in field microcosms made of polyethylene cylinders (height 10 cm, Ø 15 cm) covered with 50-µm mesh at the top and bottom to allow passage of water, but prevent entry of natural litter fall from above, and losses of small litter particles at the bottom. Two windows (5 × 18 cm) were cut into the cylinders and covered with 50 µm, 1 mm or 5 mm mesh to provide access to decomposer communities differing in body size. Windows were cut close to the bottom of the cylinders to ensure decomposers had access to a continuous layer of litter outside and inside the microcosms. An additional 1.5 cm plastic ring of the same diameter as the cylindrical microcosms was attached at the bottom of the microcosm which made it possible to push the microcosms gently into the top soil (to a depth of 1.5 cm). This held the terrestrial microcosms well in place while the bottom mesh was in intimate contact with the soil surface. In cases where pushing the microcosms into the soil was difficult (e.g. in the tropical forest with dense superficial tree roots), the 1.5 cm tall rings to position the microcosms were fitted with a separate plastic or metal ring before placing the microcosms. Microcosms were separated from each other by at least 50 cm. They were randomly distributed within blocks established at least 20 m apart from each other.

Sample harvest and processing

We removed the decomposing litter of all species from the field when 40-50% of the initial litter mass of the fastest decomposing species was remaining. As a consequence, the duration of litter decomposition varied among locations and ecosystem types (Extended Data Table 9). This

procedure ensured that very similar decomposition stages were sampled at all sites, facilitating meaningful comparisons of decomposition rates and litter diversity effects. All litter was separated to species immediately after litter retrieval. Litter recovered from the streams was gently washed to remove any adhering material and invertebrates. Litter from the terrestrial sites was cleaned by gently brushing off any dirt without using water to prevent leaching of nutrients. Litter was then dried at 65 °C for 48 hours. A correction factor was used to convert initial litter mass (weighed after drying at 40 °C) to final dry mass based on 10 randomly selected samples per litter type that were successively dried and weighed in the laboratory at 40 and 65 °C.

Litter C and N loss

Initial C and N concentrations of each of the 20 individual litter types were determined from five random samples. Final C and N concentrations after retrieval of the litter from the field were also measured for each individually sorted litter type from each microcosm. This resulted in a total of 5400 samples to calculate percent C and N loss for each litter type in the various treatments. Following the determination of litter dry mass, all initial and final samples were ground with a ball mill (Retsch PM 400, Hahn, Germany) to a fine homogenous powder. Subsamples of 3 mg were analysed for C and N concentrations using a CHN elemental analyser (Flash EA1112 Series, ThermoFinnigan, Milan, Italy). C and N loss (%) from litter during field exposure was calculated as $[(M_i \times CN_i)-(M_f \times CN_f)] / (M_i \times CN_i) \times 100$ where M_i and M_f is the initial and final litter dry mass, respectively, and CN_i and CN_f is the initial and final C or N concentration (% of litter dry mass). C loss (%) rather than total litter mass loss allowed us to correct for any possible inorganic contamination of the litter retrieved from the field.

Analyses of diversity effects and statistical models

Net diversity effects, comprising complementarity and selection effects, were calculated in species mixtures for both C and N loss[20]. The net diversity effect was calculated as the sum of complementarity and selection effects and provides a contrast of the actual C and N loss observed for mixtures of plant functional types with that expected based on C and N loss measured in single-species treatments. It represents the sum of synergistic or antagonistic interactions (i.e. complementarity effects) and those due to the presence of a dominant species (i.e. selection effects). Data were square-root transformed (keeping the original negative and positive signs for the transformed values) to meet assumptions for the analysis of variance of net diversity, complementarity and selection effects (see details below).

Analysis of variance models based on sequential sums of squares (Type I) were used to assess the effects of diversity (richness of plant litter functional types or presence/absence of a given functional type and its interaction with other functional types), completeness of the decomposer community (contrast from small to large mesh size), location across the latitudinal gradient, and ecosystem type (terrestrial versus aquatic) on percent C loss and percent N loss. To ensure meaningful comparisons across locations, several standardisation methods were tested to remove any variation associated with the differences in incubation length. These included standardising relative to: 1) a standard litter type of a non-native plant, *Ailanthus altissima*, that decomposed at all locations during the experiments, 2) the overall mean per mesh size across locations and 3) the mean per mesh size of the rapidly decomposing functional type across locations. Since results were consistent irrespective of standardisation, the final model is presented on non-standardised data.

Model terms were fitted to account for the dependency between richness of plant litter functional types (FT) and FT composition (presence/absence and interactions of functional types). First, FT composition was partitioned into a contrast for richness and residual FT composition (Table 1, Extended Data Table 5). Second, as shown in Extended Data Table 7, we omitted the

richness term and instead resolved the FT composition into a full factorial analysis with contrasts for functional type presence/absence and interactions. In this model, decomposer community was fitted as a log-linear contrast (small to large mesh size expressed as the log of the mesh size of the microcosms, which produced a linear relationship of the three mesh sizes). We also removed all other non-significant interaction terms in multiple successive model-fitting steps. These two alternative analyses reflect different partitioning of the FT composition term into contrasts; they allowed us to compare the explanatory power of the richness contrast with the presence/absence contrasts. A perfect linear richness effect would be found if all presence/absence contrasts had equal coefficients and did not interact. In this case, the mean squares or the richness effect with only one degree of freedom would be much larger than that of the combined mean squares of the presence/absence main effects of the four litter types with four degrees of freedom. In both models, the terms location and ecosystem type were tested at the block level. All other terms were tested against the residuals.

A similar analysis of variance approach was used to test independently for effects of these same factors on complementarity and selection effects as well as on net diversity effects (Extended Data Table 3). In a separate analysis of variance (Extended Data Table 8), we also tested whether the net loss of the total amount of N relative to the initial amount of N differed when litter of particular plant functional types (e.g. rapidly decomposing litter and litter of N-fixing plants) decomposed together as opposed to decomposing separately, which we interpreted as an indication of N-transfer between litter species. Location and ecosystem type were also included in this analysis. All statistical analyses were performed with the software R, version 2.8.0.

Extended discussion on litter N transfer

Although our data are suggestive that N was transferred from litter of N-fixing plants to rapidly decomposing litter, alternative mechanisms cannot be entirely ruled out. In particular, N incorporated into decomposing litter can originate not only from anothe,r co-occurring litter type, but also from the N pool in soil or stream water, or from microbial N fixation[32]. However, N transfer from such alternative N sources does not readily explain the concomitant reciprocal changes we observed between litter of the N-fixing plants and the rapidly decomposing litter types. Moreover, the idea that N transfer occurred between the two litter types is further supported by a positive net diversity effect on C loss that we observed only when these two particular litter functional types were both present (Extended Data Table 3). Additional support for our interpretation comes from two ^{15}N tracer studies in microcosms with tropical[28] and temperate forest litter[33], which serve as a proof of principle that active biological transfer of N through microorganisms, particularly saprotrophic fungi, can indeed occur. Our large-scale field experiment suggests that this phenomenon might be widespread across terrestrial and aquatic ecosystems and across a wide variety of forest types and climatic conditions.

Originally, it had been hypothesized that N transfer is driven by a gradient in N concentration between litter types[4,5], the rationale being that the element limiting decomposition rate is N. However, the scenario now unfolding from our experiment (Fig. 3) and the recent isotope tracer studies in laboratory conditions[28,33] is that N transfer is stoichiometrically controlled. The crucial determinant defining the gradient along which N will be transferred in litter mixtures appears to be the demand of N relative to C availability (and possibly that of other elements critical for decomposer growth), rather than differences in N concentration *per se*. High C quality of litter favours rapid microbial growth, which in turn entails high demands for N (and other nutrients), resulting in N acquisition from neighboring nutrient pools. In extreme cases, N

source litter may even have lower N concentrations than sink litter[32], provided that the C quality of both litter types is sufficiently different. In accordance with this mechanism, decomposition of recalcitrant litter types in our study (slowly-decomposing and evergreen plant functional types) did not profit from the presence of litter from N-fixing plant species (Extended Data Table 7), although those recalcitrant litter types had similarly low or lower initial N concentrations than the consistently benefiting rapidly decomposing litter species.

Additional References

31. Makkonen, M. *et al.* Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecol. Lett.* **15**, 1033-1041 (2012).

32. Vitousek, P.M. & Hobbie, S. Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. *Ecology* **81**, 2366-2376 (2000).

33. Lummer, D., Scheu, S. & Butenschoen, O. Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. *Oikos* **121,** 1649-1655 (2012).

34. Lepori, F. & Malmqvist, B. Deterministic control on community assembly peaks at intermediate levels of disturbance. *Oikos* **118**, 471-479 (2009).

Legends for Extended Data

Extended Data Table 1 | Characteristics of aquatic and terrestrial ecosystems at five widely dispersed locations

Footnotes:

*Means were calculated based on 10-year records between 1998 and 2008 from the closest possible meteorological station.

†Soluble Reactive Phosphorus ≈ ortho-phosphate

‡Data courtesy of K. Bishop & P.-E. Mellander.

§Soil microbial biomass (C_{mic}), soil C and soil N are expressed on a dry mass basis.

Extended Data Table 2 | Native tree species corresponding to four functional types for which leaf litter decomposition was studied (top), and quality traits associated with decomposition that vary among these four functional types (bottom)

Footnotes:

*All data in percent dry mass (mean ± SE, n=5). Methods are described in detail in Makkonen et al. (2012)[31].

Extended Data Table 3 | Results of analyses of variance testing for main effects and interactions on the net diversity effect (NE), complementarity effect (CE) and selection effect (SE) for C loss (top) and N loss (bottom) from decomposing leaf litter*

Footnotes:

*Interaction terms omitted from the final model are not significant for any of the three response variables.

†Location, ecosystem type and their interaction were tested against block rather than against the residual.

Extended Data Table 4 | Characteristics of stream macroinvertebrate communities at the five locations*, including the mean density of detritivores and their main invertebrate predators, along with total taxon richness of detritivores and predators, and the mean proportion of Plecoptera, Trichoptera and *Gammarus* as a percentage of total detritivore abundance (mean ± one standard deviation)

Footnotes:

*All samples were collected using a 500 µm mesh net, and at the same time of year as the main experiment (though in different years in some cases). Specific sampling protocols differed among locations, with all density standardized to number of individuals per meter squared:
Subarctic: Six replicate kick samples each from an area of 1 m × 0.35 m for one minute. Sampled during Autumn 2006. Identification mostly to species level, from Lepori & Malmqvist (2009)[34]. **Boreal:** Four replicate Surber samples per year for three years, with a quadrat size of 0.25 × 0.5 m. Sampled during Autumn 2010-12. Identification mostly to species level. M^{c}Kie B.G. & Hoffsten P-O. *Unpublished data.***Temperate:** Five replicate sweep net samples each from an area of 0.3 × 5m. Sampled Autumn 1992. Identification mostly to species level. Nijboer R. De Springendalse Beek. Macrofaunagemeeenschappen in de periode 1970-1995 (1999).
Mediterranean: Five replicate Surber samples, with a quadrat size of 0.33 × 0.31 m. Sampled January 2014. Identification mostly to family level. Chauvet, E. & Lamothe, S. *Unpublished data.***Tropical:** Ten replicate natural leaf packs (fist-sized handfuls of leaves picked from the stream bed) from each of seven streams. Abundances per leaf pack were converted to densities based on standardized visual estimates of stream bed litter cover. Sampled May 2007. Identification mostly to family level. Bruder, A., Schindler, M., Moretti, M.S. & Gessner, M.O. *Unpublished data.*

†Detritivore community composition data does not sum to 100% at all locations, due to the presence of additional dipteran (Tipulidae), lepidopteran (Pyralidae) and crustacean shredders (Asellidae) at the Temperate site, and tipulid and pyralid shredders at the Tropical site.

‡The caddisfly *Micrasema* (Brachycentridae) was common at the Mediterranean site, but was small and not regarded as a shredder.

Extended Data Table 5 | Full model output of the relative contributions of variance associated with diversity and sites expressed in percent sums of squares (% SS) to explain C

and N loss in a large-scale leaf litter decomposition experiment (P<0.001[***], P<0.01[**], P<0.05[*])

Footnotes:

[†]Plant species were selected to represent the same four functional types (FT) at each location (N-fixing, evergreen, rapidly or slowly decomposing deciduous trees/shrubs). Litter functional type richness (linear contrast) and litter diversity (factorial contrast) were fitted prior to litter FT compositions.

[‡]See Table 1 for footnote.

Extended Data Table 6 | Characteristics of soil fauna communities[*] at the five locations, including density, total taxon richness[†] and the proportion of dominant taxa[‡] as a percentage of total community abundance (mean ± one standard deviation)

Footnotes:

[*]Communities are split in mesofauna and macrofauna reflecting an increase in body size which relates to mesh size differences in the field microcosms.

[†]Taxon richness is based on the number of observed families.

[‡]Dominant taxa is based on a lower taxonomic resolution, mainly order or class level. Community composition data does not always sum to 100% at all locations, due to the presence of additional taxa.

[§]All samples were collected at the end of the growing season in 2008 (Subarctic and Boreal late September; Temperate and Mediterranean October, Tropical early December). Eight Kempson cores (diameter 21 cm) and eight Macfayden cores (diameter 5 cm) were taken at each field site. The reported data is based on extraction of the whole soil core of nine cm height, including the litter layer. Soil arthropods were extracted, counted and identified all to the highest possible taxonomic level (families). Butenschoen, O & Scheu, S. *Unpublished data.*

Extended Data Table 7 | Analysis of variance testing for the presence of each of four plant

functional types, completeness of the decomposer community, location, ecosystem type (terrestrial vs. aquatic) and their interactions on total litter C (top) and N loss (bottom) (all terms included in the final model shown are significant at *P* <0.05)

Footnotes:

*Decomposer community was fitted as a log linear contrast and not a factorial contrast (as shown in Table 1).

†Location, ecosystem type and their interaction were tested against block rather than against the residual.

Extended Data Table 8 | Analysis of variance testing the proportional change in total litter N content when two particular plant functional types (N fixing and rapidly decomposing) decomposed together in two-species combination as opposed to decomposing individually

Footnotes:

*A significant difference of the mixture × functional type interaction is taken as an indication of N-transfer between litter species.

†Location, ecosystem type and their interaction were also included as factors in this analysis and were tested against block rather than the residuals.

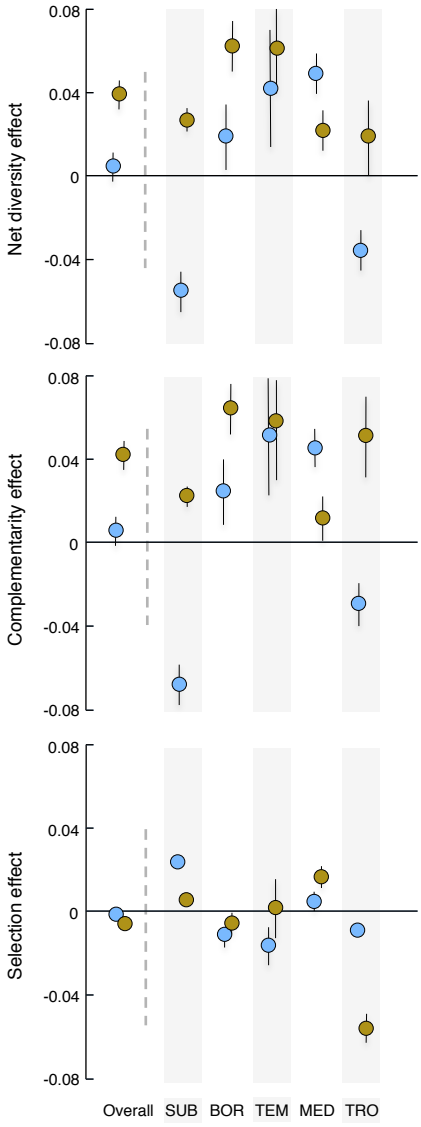
Extended Data Table 9 | Experimental duration and richness of naturally occurring local litter species in terrestrial and aquatic ecosystems at each of five widely dispersed locations

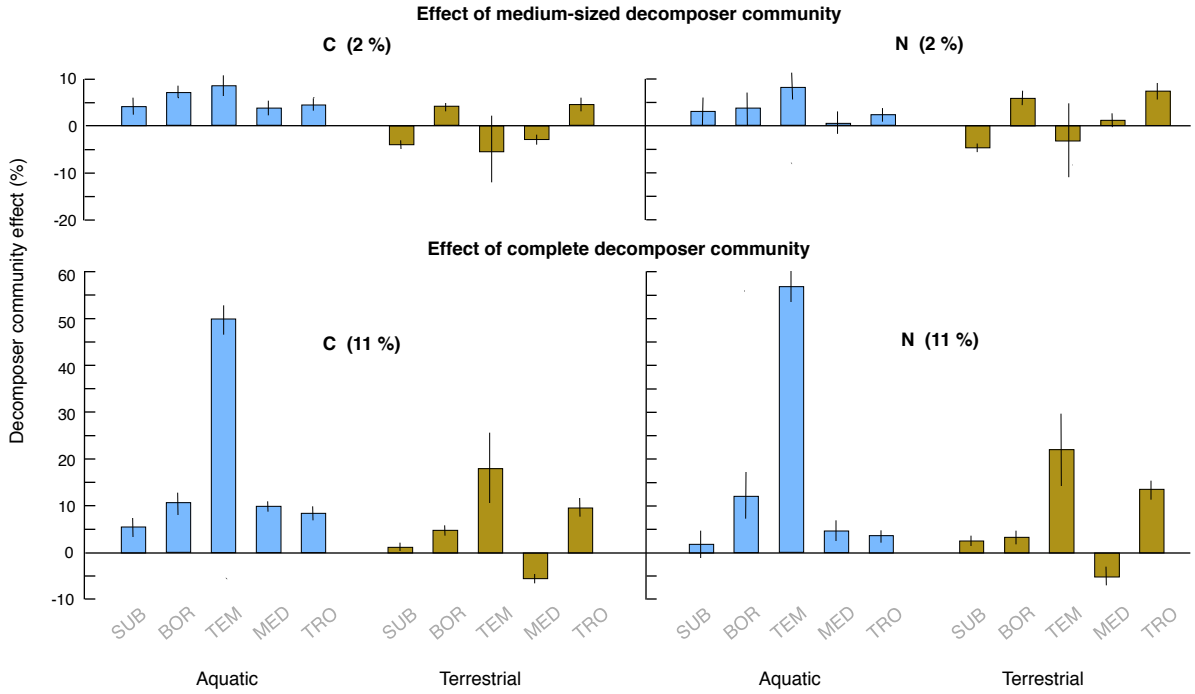
*Incubation dates differed across ecosystem types and locations to ensure 40-50% mass remaining of the most rapidly decomposing litter at the time of sampling, thus allowing

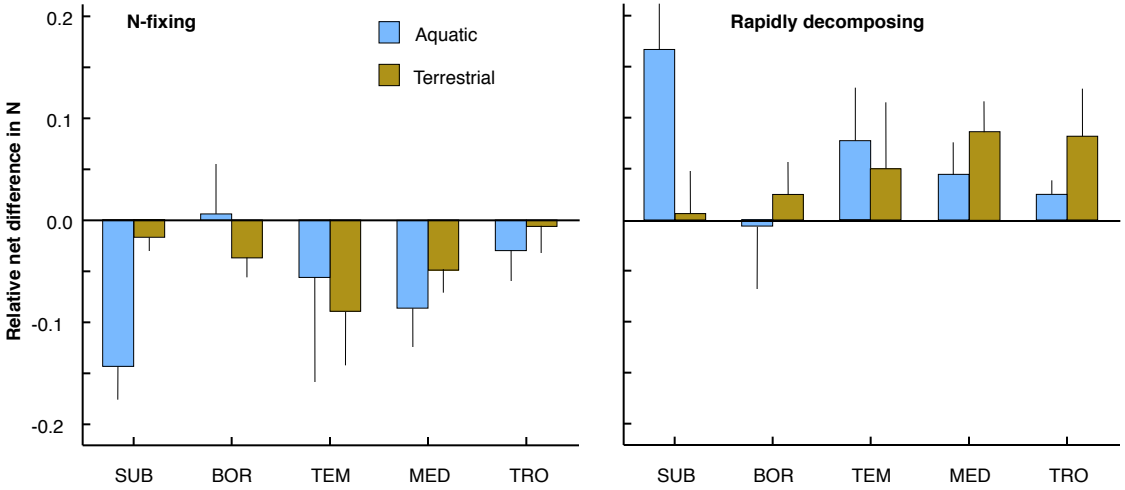
comparisons at similar decomposition stages.

[†]Mean species richness counts of naturally occurring litter in five randomly sampled plots that were the size of microcosms (Ø = 15 cm) in each of the five experimental blocks

Extended Data Figure 1 | Net diversity, complementarity and selection effects of all plant litter mixtures for N loss. Net diversity effects are the deviations from the expected means based on N loss measured from single litter species. Blue and brown circles show mean effects (±SE) in forest streams and on forest floors, respectively, in subarctic (SUB), boreal (BOR), temperate (TEM), Mediterranean (MED) and tropical (TRO) locations. Each symbol shows the means effect per ecosystem type (i.e. aquatic versus terrestrial) calculated across the three types of decomposer communities (n=825 litter mixtures for the overall mean per ecosystem type, or n=165 litter mixtures per location in each of the two ecosystem types).


Net diversity effect
Complementarity effect
Selection effect
0.08
0.04
0
-0.04
-0.08
Overall
SUB
BOR
TEM
MED
TRO


Effect of medium-sized decomposer community
C (2 %)
N (2 %)
Effect of complete decomposer community
C (11 %)
N (11 %)
Decomposer community effect (%)
10
0
-10
-20
60
50
40
30
20
10
0
-10
SUB
BOR
TEM
MED
TRO
Aquatic
Terrestrial


N-fixing
Rapidly decomposing
Aquatic
Terrestrial
Relative net difference in N
0.2
0.1
0.0
-0.1
-0.2
SUB
BOR
TEM
MED
TRO
SUB
BOR
TEM
MED
TRO