

SUPPLEMENTARY MATERIAL 4 - MATERIALS AND METHODS

Study area

The study sites were located within the Córdoba Province (Argentina) across four systems; Sub-Andean *Polylepis australis* forests, Mountain Chaco *Lithraea molleoides* forests, Espinal *Prosopis caldenia* forests, and Arid Chaco *Aspidosperma quebracho-blanco* forests (Figure 1-Main text). For details on climate, location, and vegetation characteristics see Supplementary Material 2. Within each ecosystem, we selected 3 stands of forest and 3 stands of treeless physiognomies (grasslands or shrublands, which were the result of a transition from a forest that was logged and/or burnt, followed by chronic grazing). In each stand, we established a 20x20 m square within which we made a floristic *relevé* and we recorded vascular plants species cover following Braun-Blanquet abundance-cover scale (Kent and Coker 1992). A more detailed description of the vegetation characteristics can be found in Vaieretti et al. (2021).

Litter and soil samples

In each stand, we collected a compound sample of naturally mixed above-ground litter (leaf and stems) in four quadrats of 25x25 cm, in two dates within the peak of litterfall (July and September) following a procedure similar to that of other experiments (Fortunel et al. 2009; Vaieretti et al. 2013). The litter was air-dried and stored in paper bags until the construction of the different litterbags for experiments 2 and 3 (below). We sent a subsample of each litter mixtures to the National Institute of Agricultural Technology (INTA, Bariloche, Argentina) where they determined total nitrogen (N) and phosphorous (P) with an Autoanalyser (RFA 300-Alpkem, Wilsonville, O.R., USA), following O'Neill and Webb (1970) and Westerman (1990), respectively. On the same sample, INTA Bariloche determined lignin, cellulose, and hemicellulose content using the technique of Goering and Van Soest (1970). We estimated total carbon (C) as 50% of ash-free biomass (Gallardo and Merino 1993; McClaugherty et al. 1985).

To characterise the soils, in each stand, we collected a soil compound sample of the upper horizon (0-15 cm) where we measured: soil texture (Day 1965), pH (Thomas 1996), organic C (%) by Walkley-Black technique (Nelson and Sommers 1996), total N (%) by Kjeldahl technique (Bremner 1996), extractable P (ppm) by Bray & Kurtz N°1 technique (Kuo 1996), cationic exchange capacity (CEC meq/100 g) and cations (Ca⁺⁺, Na⁺, Mg⁺⁺, and K⁺ meq/100 g) (Sumner and Miller 1996).

Decomposition experiments

To evaluate the local environment for decomposers ("standard substrates decomposition", Experiment 1 - Figure 3) we incubated, at each stand, two standard substrates (Binkley 1984; O'lear et al. 1996; Cuchietti

et al. 2017; Swan et al. 2021) which have been widely used because they provide insights about the effect of the local environment on decomposition, independently of the substrate quality (Jenny et al. 1949; Tsarik 1975; Piene and Van Cleve 1978; Orwin et al. 2006; Dujukic et al. 2018). The standard substrates selected were filter paper and popsicle wood sticks (Berg and Lawkosky 2005; Vaieretti et al. 2010). With those materials, we made 30 litterbags (subsamples) of 0.3 mm mesh nylon to be incubated in each stand, 15 of them were filled with 5 g of the air-dried popsicle wood sticks and 15 of them were filled with 2 g of air-dried filter paper. Although the mesh size used reduces access to meso- and macro-fauna, it helps to prevent loss of material during manipulation, and we have previously tested that its use does not affect the decomposition pattern significantly (Cornelissen et al. 1999; Vaieretti et al. 2010). We retrieved a third of the bags (5 of each standard material) after four months (August), another third after 8 months (December), and the last litter bags after 12 months (April). Unfortunately, a high proportion of the litterbags were missing for the last retrieval date so we are presenting the decomposition after 8 months for the comparison with the other variables. Also, considering that the filter paper represented a highly decomposable material (it reached an average 94.2% mass loss in the Espinal and 78.5% mass loss in the Subandean systems, after 8 months of incubation, representing the extremes values found) while popsicle woodsticks represented a lowly decomposable material (it reached an average 45.6% mass loss in the Espinal and 10.9% mass loss in the Subandean systems, after 8 months of incubation) we are presenting the averaged the decomposition of these standard substrates to better compare it with the *in situ* mixtures decomposition that also included high and low decomposition substrates.

To evaluate the decomposability of the litter produced in each stand (“mixtures decomposability”, Experiment 2-Figure 3) we made 20 litterbags (subsamples) using 0.3 mm mesh nylon, which we filled with 1 g of air-dried naturally mixed litter collected from each stand, and we incubated the litter bags from all stands simultaneously in a common garden (Cornelissen et al. 1999; Pérez-Harguindeguy et al. 2013). Although the small mesh size could reduce access to soil fauna (Seastedt 1984; Bradford et al. 2002), it helps to prevent loss of material during manipulation (Pérez-Harguindeguy et al. 2013). Decomposability measured in common garden incubation is an expression of the quality of different materials as a substrate for microorganisms (Pérez-Harguindeguy et al. 2013). The incubation started at the end of the summer (February) and lasted 90 days. We retrieved half of the litterbags after 40 days to evaluate the course of decomposition.

Finally, to evaluate the decomposition of naturally mixed litter in the environment from which it has been produced (“*in situ* litter mixture decomposition”, Experiment 3, Figure 3) we built 30 litter bags (subsamples) of 0.3 mm mesh nylon (‘thin mesh’), which we filled with 5 g of the air-dried naturally

mixed litter from each stand, and we incubated the litter bags from each stand within the same stand where litter has been collected (Fortunel et al. 2009; Vaieretti et al. 2013). These litter bags were incubated for a whole year starting at the beginning of Autumn (March-April), placed side by side with the litter bags filled with standard materials. As with the standard materials, we retrieved a third of the bags after four months (August), another third after 8 months (December), and the last litter bags after 12 months (April). To be able to compare the decomposition pattern with that of the standard materials we are presenting the results of the decomposition after 8 months (March-December), we checked that the pattern after 12 months remained the same. With the data of *in situ* mixtures' decomposition after 8 months, we also calculated the decomposition constant $k = -\ln(M_t/M_0)/t$ (where M_t = final litter mass and M_0 = initial litter mass and t = time in days) to give a common estimator of the systems' rate of decomposition that can be compared to those found in other studies and other systems.

For the three experiments, after each incubation period, we stored the bags at -14 °C until processing. To determine the mass loss of each subsample of naturally mixed litter and standard material, once the decomposition bags were defrosted we removed adhering soil, soil fauna, and other extraneous materials by brushing or swiftly rinsing with water. We then oven-dried the content of the cleaned bags for at least 48 h at 60 °C, and then weighed them. To estimate initial dry mass before incubation, we calculated air-dried water content by drying the material in sub-samples at 60 °C for 48 h. Water content (%) was calculated from the weight loss in sub-samples after drying. We estimated the decomposition of each subsample as the percentage of dry weight loss at the end of the incubation period (Pérez-Harguindeguy et al. 2013). We obtained one value of mixtures decomposability, standard materials decomposition, and *in situ* mixtures decomposition per stand at the end of incubation by averaging the dry weight loss of all subsamples retrieved.

Data analysis

To compare litter mixtures' decomposability, decomposition of standard substrates, and *in situ* litter mixtures decomposition, we used general linear models. System (i.e., Sub-Andean, Mountain Chaco, Espinal, and Arid Chaco), physiognomy (i.e., forest and treeless), and their interactions were considered fixed factors. When the interactions were not significant, they were eliminated from the models. When the system, the physiognomy, or their interaction between system and physiognomy were significant, we performed a pairwise comparison using Tukey test with the emmeans R package (Lenth et al. 2020) to test if the previously observed differences were caused by the system or by the physiognomy. Additionally, we performed a multiple regression across all sites (n=26) with *in situ* litter decomposition as the

dependent variable and the standard material decomposition and the litter mixtures decomposability as independent variables. All statistical analyses were conducted using R 3.5.0 (R core team 2018).

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