Appendix A

"Blooming" of litter-mixing effects: The role of flower and leaf litter interactions on decomposition in terrestrial and aquatic ecosystems

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Extended methods

1 Species used

Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex. S. Moore individuals, similar to other species in the Bignoniaceae family, undergo a massive synchronous flowering preceded by the abscission of leaves, creating a real-world scenario for the interaction between the two types of litter in nature (Fig. A1; Lorenzi 1992; Batalha and Mantovani 2001).

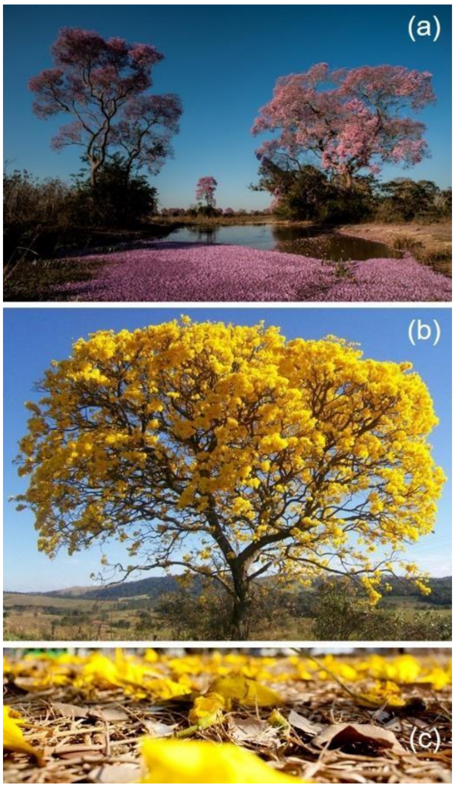

Figure A1: Photos depicting variants of the trumpet tree in bloom of genus Tabebuia (Figures a and b). The mass-concentrated-flowering phenology, exhibited by most species of the genus Tabebuia right after leaf shedding, generates a leaf and flower litterfall chronosequence in both aquatic (Figure a; individual of *Tabebuia impetiginosa* (Mart. ex DC.) Standl.) and terrestrial (Figure b; individual of *T. aurea*) systems. This phenological chronosequence results in a specific positioning of leaf and flower litter on the litter layer beneath *T. aurea*'s individuals (Figure c). Photo by Rafael D. Guariento.

2 Experimental design and setup

We used an additive rather than a substitutive design. Additive designs maintain a constant biomass for a given species in monocultures and mixtures; thus, the total biomass in mixtures is the sum of the species biomass in their respective monocultures. By contrast, in substitutive designs, the total biomass in a mixture is equal to the total biomass in the monoculture. Thus, the biomass of a given species in a mixture is equal to the total biomass divided by the number of species in the mixture (Jolliffe, 2000). The choice of experimental design depends on how the biomass/abundance of a species varies across species richness gradients in nature (Schmid et al., 2002). For example, in diversity experiments, it is assumed that the biomass of litter or the number of individuals per species decreases with species richness because if the amount of resources is finite, the increase in species number necessarily reflects a decrease in the biomass/abundance of each coexisting species (Garnier et al., 1997). However, this rationale does not find any logical support for the mixing pattern of flower and leaf litter originating from the same species in nature, justifying the use of an additive experimental design. Furthermore, additive designs are simpler for comparing how mixing litter affects the decomposition of focal species or litter types. This is because the biomass of a focal litter is the same in both its monoculture and in the litter mixture. Therefore, any observed differences in the decomposition of a focal species or litter in the litter mixture compared to its monoculture are due to its interaction with other litter types/species in the mixture.

Table A1: Summary of the additive experimental design used to evaluate the effect of flower and leaf litter mixtures on decomposition rates in both terrestrial and aquatic experiments. The total mass of flowers and leaf litter added to each microcosm varied at 9 levels in monocultures and their corresponding mixtures. In litter mixtures, the total litter mass per microcosm was 3g (dry weight), while the flower:leaf litter mass proportion ranged from 0.1 to 0.9. In the terrestrial environment, the monocultures and mixtures were replicated 10 times, totaling 270 microcosms. In the aquatic environment, the monocultures were replicated three times and mixtures were replicated six times, with a total of 108 microcosms. The entire experimental design encompassed 378 microcosms.

Proportion of flower litter mass in mixtures	Total litter biomass in mixtures (g)	Flower litter biomass in monocultures and their respective mixtures (g)	Leaf litter biomass in monocultures and their respective mixtures (g)
0.1	3.0	0.3	2.7
0.2	3.0	0.6	2.4
0.3	3.0	0.9	2.1
0.4	3.0	1.2	1.8
0.5	3.0	1.5	1.5
0.6	3.0	1.8	1.2
0.7	3.0	2.1	0.9
0.8	3.0	2.4	0.6
0.9	3.0	2.7	0.3

3 References

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