Grouping nitrogen fixing trees into discrete functional groups based on litter decomposition rate does not make sense

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Article Info	Abstract
	Functional grouping of nitrogen fixing trees into discrete groups is a good approach to understanding their influence on ecosystem functioning in their new environment. Most of previous studies have reported faster leaf litter decomposition rates of nitrogen fixing than non-nitrogen fixing species. Meta-analysis using published data is the best way for functionally grouping of nitrogen fixing trees from non-nitrogen fixing trees based on litter decomposition rate. Meta-analysis was used for analyzing litter decomposition rate from published data. The data extracted from 5 papers and 16 species that used laboratory method and 27 papers and 41 species that used litterbag method. Leaf litter decay constant (k year^{-1}) of the nitrogen fixing trees was not different from non-nitrogen fixing trees. Initial leaf litter quality (N or C/N, lignin/N, Tannin and Phenolics) of nitrogen fixing trees in all studies was higher than non-nitrogen fixing trees. Totally, it could be highlighted that leaf litter decomposition is species dependent and functional grouping of the tree species based on nitrogen fixing ability is not reasonable, although it is apparent that the litter quality of the two groups is different.

Introduction

Nitrogen fixing ability of plants is a key trait that has large ecosystem-level consequences (Kurokawa et al., 2010). Nitrogen fixing species play an important role in ecological restoration worldwide (Zhu et al., 2016). Therefore, tree species with nitrogen fixing ability have been widely used as pioneer plants to facilitate ecological restoration in eroded and degraded ecosystems. Also several previous studies have reported the increase of productivity while maintaining soil fertility, enhancing soil organic carbon sequestration and accelerating nutrient cycling by admixture of nitrogen fixing trees to *Eucalyptus* (Forrester et al., 2006; 2013; le Maire et al., 2013) and *Popolus* (Sayyad et al., 2006) pure plantations (Wu et al., 2014). However, other works reported the similar, even lower total stand productivity in mixed-species plantations of *Eucalyptus* with nitrogen fixing trees, compared to *Eucalyptus* pure plantations (Tang et al., 2013). Moreover, litter in mixtures with nitrogen fixating species does not necessarily decay faster than monoculture litters of non- nitrogen fixation species (Wu et al., 2014). Grouping together species with similar traits into functional groups can help us to generalize the influence of exotic plants in new environment (Yelenik et al., 2007; Liao et al., 2008) and predict their behavior in these ecosystems.

Leaf litter decomposition is a fundamental ecosystem process that regulates humus formation, ecosystem carbon storage, nutrient cycling (Cizungu et al., 2014; Zhu et al., 2016) and nutrient availability (Wang et al., 2007; Tang et al., 2013). Therefore plants with recalcitrant litter, immobilize nutrients in organic pools for long time, whereas plants with labile litter, release rapidly available nutrients for species uptake (Guo and Sims 2001; Rothstern et al., 2004). Litter decomposition rate is determined by functional tree type (Wedderburn and Carter, 1999; Swarnalatha and Reddy, 2011; Bachega et al., 2016; Buettel et al., 2019). Previous studies have reported faster leaf litter decomposition rates of nitrogen fixing than non-nitrogen fixing species (Aerts and Chapin, 2000), supporting the separation of these as functional groups. In opposition Yelenik et al., (2007) reported vast differences within nitrogen fixing group that revealed, not all nitrogen fixing species exert similar effects. Surprisingly, in a recent research Sayad et al., (2015) found that leaf litter of non-nitrogen fixing trees decompose faster than nitrogen fixing trees. Whereas, Kurokawa et al., (2010) and Lang et al., (2009) did not find the two groups different in leaf litter decomposition rate. As many of previous studies (except some e.g. Kurokawa et al., (2010) and Lang et al., (2009)) only compared one species in each group, we could not rely on their results in functional grouping of nitrogen fixing trees from non- nitrogen fixing trees. On the other hand, most of the researchers found that leaf litter of nitrogen fixing species had higher nitrogen concentrations and lower C: N than did non- nitrogen fixing species (Peltzer et al., 2009; Kurokawa et. al. 2010; Sayad et al., 2015). Liao et al., (2008) also reported greater C and N cycle under nitrogen fixing species (in line with many previous researches, e.g. Binkley and Giardina, 1998) through meta-analysis. If leaf litter quality of nitrogen fixing species apparently were different with those of non- nitrogen-fixing species, so we could predict leaf litter decomposition rate of tree species based on their nitrogen fixation ability (Kurokawa et al., 2010). Hence, more researches with numbers of species within each group are still necessary for better understanding of

functional grouping of the trees based on leaf litter decomposition rate.

The meta-analytical method is a highly important tool that is widely used by ecologists to generate more powerful generalizations about the issues confronting us. This method generally has advantages over narrative or quantitative reviews, which lack sampling rigor and robust statistical methods (Wang et al., 2013). To date, however, a literature review of the grouping nitrogen fixing trees based on leaf litter decomposition via meta-analysis is unavailable, which limits our understanding the influence of nitrogen fixing trees on ecosystem. Until now, most researchers considered limited numbers of functional groups (Wedderburn and Carter, 1999). In order to decrease the variability of leaf litter decomposition rate in each group we limited the selection of the species from published data to the same genus as Sayad et al., (2015) used. Therefore, 32 published papers were used to compare the leaf litter decomposition constant of nitrogen fixing trees and non-nitrogen fixing trees. Our aim was to compare leaf litter decomposition rate of nitrogen fixing tree with that of non-nitrogen fixing tree.

Materials and methods
Data extraction
Data were extracted from published papers. Literature searches of primary research in published, peer reviewed journal sources were performed using different electronic database including: Elsevier, Springer, Wiley and Google search. We had tried to do our literature survey as inclusive as possible. The search parameters were limited to papers, which titles, abstracts, and keywords referred to litter decomposition, decay, nitrogen fixing trees and non-nitrogen fixing trees. Of the papers retrieved by the search, those that had a nitrogen fixing trees or a non-nitrogen fixing trees were selected. As leaf litter decomposition were studied using two different methods, litterbag and laboratory, the final data set containing 5 papers and 16 species that used laboratory method (Table 1), 25 papers and 39 species that used litterbag method (Table 2). A data thief software (GetData Graph Digitizer) was

used to extract data from figures. The time of all extracted leaf litter decomposition constant converted to one year. In addition, leaf litter decomposition constant were calculated for the researches that reported mass loss or remaining mass. We used the leaf litter decay constant (k) of 57 species of the two functional groups from the published papers. In order to control the variability of leaf litter decomposition among species we decide to limit the species selection to some genus from each of the two groups. Therefore, we selected the species from the same genus that were used by Sayad et el. (2015). The other factor that we considered to reduce the variability of leaf litter decomposition among species was climate, which has strong influence on leaf litter decomposition. Consequently, along with limitation in our selections we could control the covariate the influence leaf litter decomposition of species. Initial leaf litter quality of the nitrogen fixing trees and non-nitrogen fixing trees also were extracted from the papers that at least compared one tree species of each group (Table 3).

Statistical analyses
We performed mixed model ANOVA to determine the impact of functional groups on leaf litter decay constant with functional groups as a fixed effect and laboratory as a random effect for the data of Table 1, as litter decomposition constant determined in different conditions in the laboratory. For the data of Table 2 that litter decomposition constant determined by litterbag method in the field we performed mixed model ANOVA to determine the impact of functional groups on leaf litter decay constant with functional groups as a fixed effect and climate as a random effect, as the climates of the original experiments were different. For the data of Table 3 because of higher leaf litter quality of all nitrogen fixing trees than non-nitrogen fixing trees no analysis was used. All the analyses have done with SPSS

Results
We demonstrate that leaf litter decay constant (k year^{-1}) of the nitrogen fixing trees was not different from non-nitrogen fixing trees. Surprisingly the results of the two meta-analyses showed higher leaf litter decay constant for nitrogen fixing trees than non-nitrogen fixing trees, although it was not significantly different (Table 1 and Table 2). The review of the initial leaf litter quality of the two groups indicated that in all studies, nitrogen fixing trees had higher initial leaf litter quality (higher N or lower C/N, lignin/N, Tannin and Phenolics) than non-nitrogen fixing trees (Table 3).

Table 1. Overview of annual laboratory leaf litter decay constant (k) studies in nitrogen and non-nitrogen fixing trees included in meta-analysis.

Laboratory	Species	k	FT[b]	Reference
1	*Acacia pravissima*	0.29	Yes	Kurokawa et al., (2010)
	Acacia sp	0.32	Yes	Kurokawa et al., (2010)
	Acacia mearnsii	0.35	Yes	Kurokawa et al., (2010)
	Acacia dealbutum	0.34	Yes	Kurokawa et al., (2010)
	Populus alba	0.44	No	Kurokawa et al., (2010)
	Populus deltoids	0.45	No	Kurokawa et al., (2010)
2	*Eucalyptus camaldulensis*	1.53	No	Hasanuzzaman and Hossain, (2014)
	Acacia auriculiformis	0.67	Yes	Hasanuzzaman and Hossain, (2014)
	Dalbergia sissoo	1.14	Yes	Hasanuzzaman and Hossain, (2014)
3	*Acacia mangium*	0.62	Yes	Li et al., (2001)
	Acacia auriculaeformis	0.66	Yes	Li et al., (2001)
	Eucalyptus citriodora	0.84	No	Li et al., (2001)
4	*Eucalyptus*	4.57	No	Bernhard-Reversat, (1999)
	Acacia auriculiformis	3.43	Yes	Bernhard-Reversat, (1999)
5	*Eucalyptus globules*	1.2	No	Xiang and Bauhus, (2007)
	Acacia mearnsii	1.75	Yes	Xiang and Bauhus, (2007)
	Non--nitrogen Fixing Trees	1.51		
	Nitrogen Fixing Trees	0.96		
	Functional groups effect	ns[a]		

[a] ns = treatment effect not significant at 0.05 .
[b] NFT=Nitrogen Fixing Tree

Table 2. Overview of annual litterbag leaf litter decay constant (k) studies in nitrogen and non- nitrogen fixing trees included in meta-analysis.

Climate	Species	Mesh size (mm)	K	FT[b]	Reference
Temperate	*Eucalyptus brookerana*	1	0.94	no	Guo and sims, (1999)
	Eucalyptus botryoides	1	0.35	no	Guo and sims, (2001)
	Eucalyptus botryoides	1	0.36	no	Guo and sims, (2002)
	Eucalyptus dunnii	1	0.81	no	Hernandez et al., (2009)
Tropical	*Eucalyptus sp.*	2	0.84	no	Cizungu et al., (2014)
	Eucalyptus grandis	2	0.49	no	Bachega et al., (2016)
	Acacia mangium	2	0.34	yes	Bachega et al., (2016)
	Eucalyptus urophylla	1	1.99	no	Barlow et al., (2007)
	Acacia mangium	-	0.60	yes	Santos et al., (2018)
Subtropical	*Eucalyptus grandis*	1	1.44	no	Wu et al., (2014)
	Dalbergia sissoo	1	1.50	yes	Hossain et al., (2011)
	Eucalyptus camaldulensis	1	1.55	no	Demessie et al., (2012)
	Eucalyptus golublus	1	1.09	no	Demessie et al., (2012)
	Eucalyptus camaldulensis	1	1.38	no	Demessie et al., (2012)
	Eucalyptus sp.	1	1.00	no	Reddy and Venkataiah, (1989)
	Acacia confusa	-	0.36	yes	Lin et al., (2020)
Monsoon	*Acacia nilotica*	5*5	2.37	yes	Swarnalatha and Reddy, (2011)
	Eucalyptus sp.	5*5	1.70	no	Swarnalatha and Reddy, (2011)
	Dalbergia sissoo	1	0.99	yes	Semwal et al., (2003)
	Acacia auriculiformis	0.5*2	0.56	yes	Zhu et al., (2016)
	Eucalyptus urophylla	0.5*2	0.71	no	Zhu et al., (2016)
	Acacia auriculiformis	1	0.87	yes	Dutta and Agrawal, (2001)
	Eucalyptus hybrid	1	0.69	no	Dutta and Agrawal, (2001)
	Eucalyptus tereticornis	2	1.90	no	Das and Chaturvedi, (2003)
	Eucalyptus tereticornis	1	1.95	no	Bargali, (1995)
Semiarid	*Eucalyptus camaldulensis*	1	0.52	no	Tang et al., (2013)
	Acacia auriculiformis	2	0.51	yes	Ngoran et al., (2006)
	Acacia mangium	2	0.53	yes	Ngoran et al., (2006)
	Populus euphratica	1	1.51	no	Sayad et al., (2015)
	Eucalyptus camaldulensis	1	0.90	no	Sayad et al., (2015)
	Eucalyptus microtheca	1	0.82	no	Sayad et al., (2015)
	Acacia saligna	1	0.46	yes	Sayad et al., (2015)
	Acacia stenophylla	1	0.32	yes	Sayad et al., (2015)
	Acacia salicina	1	0.96	yes	Sayad et al., (2015)
	Dalbergia sissoo	1	0.91	yes	Sayad et al., (2015)
	Eucalyptus camaldulensis	2	0.75	no	Doldoum et al., (2010)
Mediterranean	*Eucalyptus diversicolor*	1.5	0.83	no	O'Connell, (1986)
	Acacia caven	2	0.44	yes	Martínez et al., (2010)
	Eucalyptus diversicolor	3	0.31	no	O'Connell, (1988)
	Acacia melanoxylon	1	0.21	yes	Wedderburn and Carter, (1999)
	Eucalyptus nitans	1	0.29	no	Wedderburn and Carter, (1999)
	Non- nitrogen Fixing Trees		0.97		
	Nitrogen Fixing Trees		0.95		
	Functional groups effect		ns[a]		

[a] ns = treatment effect not significant at 0.05 .
[b] NFT=Nitrogen Fixing Tree

Table 3. Overview of studies that compared initial leaf litter quality in nitrogen and non-nitrogen fixing trees.

Number of non-nitrogen fixing trees	Number of nitrogen fixing trees	litter quality Criteria	result	Reference
1	1	N	Yes	Pozo et al., (1998)
1	1	C/N and lignin/N	Yes	Wedderburn and Carter, (1999)
1	2	lignin/N	Yes	Bernhard-Reversat and Schwartz, (1997)
1	1	N	Yes	Dutta and Agrawal, (2001)
1	1	N	Yes	Das and Chaturvedi, (2003)
1	1	C/N	Yes	Xiang and Bauhus, (2007)
22	19	C/N	Yes	Kurokawa et al., (2010)
2	1	C/N	Yes	Doldoum et al., (2010)
1	1	C/N	Yes	Swarnalatha and Reddy, (2011)
1	1	C/N	Yes	Tang et al., (2013)
1	1	C/N and lignin/N	Yes	MacKenzie et al., (2013)
3	4	C/N	Yes	Sayad et al., (2015)
1	1	C/N	Yes	Zhu et al., (2016)
1	1	C/N, Total tannins and total phenolics	Yes	Bachega et al., (2016)

Yes: means higher quality in nitrogen fixing trees (higher N or lower C/N, lignin/N, Tannin and Phenolics). No: means lower quality in nitrogen fixing trees (higher N or lower C/N, lignin/N, Tannin and Phenolics)

Discussion

Faster leaf litter decomposition of non-nitrogen fixing trees than nitrogen fixing trees reported by Sayad et al., (2015) was in contrary to previous findings (Knops et al., 2002; Tateno et al., 2007; Cornwell et al., 2008) that found faster leaf litter decomposition of nitrogen fixing trees. Whereas the result of our meta-analysis that is more clarifying, did not find any difference in leaf litter decomposition of the two groups. This result is in line with the results of Kurokawa et al., (2010) and Lang et al., (2009) that both compared numbers of species in each groups. Therefore, as Kurokawa et al., (2010) and Yelenik et al., (2007) concluded, we could state that between species differences were more important than their belonging to the functional groups. Hence, species identity within functional groups is more important determinant of ecosystem level impacts than functional groups. In addition, we could state that leaf litter decomposition rate is species dependent. In line with Wu et al., (2014) we also could state that it is important to select suitable trees among nitrogen fixation trees with readily decomposable litter and high rates of

nutrient cycling to rehabilitate the destroyed ecosystem.

Several studies indicated the role of leaf litter quality for enhancing decomposer activities in tropical and temperate forests (Cizungu et al., 2014). Different litter parameters like initial litter N concentration and C/N ratio have been found to be useful as predictors of the decay rate (Swarnalatha and Reddy, 2011). In the early stage of litter decomposition, N may have a positive effect on litter decomposition. However, during the later stages, when the rate of litter decomposition is dominated by the degradation of lignin and modified lignin-like humification products, N may have a negative influence on lignin degradation, leading to the production of substantial amounts of residue (Zhu et al., 2016). Previous studies showed that species of high leaf litter quality had higher decomposition rates than those of lower quality (Cizungu et al., 2014). Lack of difference between leaf litter decomposition rate of nitrogen fixing trees and non-nitrogen fixing trees is in contrary to these previous findings, as our findings showed higher leaf litter quality of nitrogen fixing trees than non- nitrogen fixing trees. This

conflict may rise from two reasons a: leaf litter quality criteria that applied in literatures might not be suitable in predicting leaf litter decomposition rate, b: most probable reason might be the presence of a covariate that influence leaf litter decomposition rate that varies between the species not two groups.

Totally, this research highlighted that leaf litter decomposition is species dependent and functional grouping of the tree species based on nitrogen fixing ability is not reasonable, although it is apparent that the litter quality of the two groups is different.

Conflict of interest
The author declares that has no conflict of interest.

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