

Letters

Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands

Arbuscular mycorrhiza represents a ubiquitous nutritional symbiosis between the roots of most terrestrial plant species and fungi of the subphylum Glomeromycotina (Spathafora *et al.*, 2016). Terrestrial habitats are unlikely to be limited in propagules of arbuscular mycorrhizal fungi (AMF), because AMF propagule densities build up fast in vegetated soil (e.g. Gould *et al.*, 1996). We start to appreciate, however, that shortages in AMF propagules are common in some habitats, such as agricultural fields subject to intensive farming (Schnoor *et al.*, 2011; Manoharan *et al.*, 2017). Forest habitats in the temperate region might also be occasionally AMF propagule limited (Veresoglou *et al.*, 2017), but to the best of our understanding this has not been shown with empirical data.

A particular feature of forest habitats is that two different strata of plants co-occur: the canopy consisting of woody plants and the understory mainly consisting of herbaceous plants. The two strata interact in various ways even though the underlying mechanisms remain mostly unknown (Sutherland *et al.*, 2013). Woody plants through intercepting light and via exploitative competition could alter germination and growth of herbaceous plants (Barbier *et al.*, 2008). However, it is unlikely that herbaceous plants can alter significantly the fitness of mature woody plants, rendering their interaction asymmetric. An underexplored factor in the way the two strata influence each other is mycorrhiza: the minority (i.e. coverage) of woody plants in Central European forests associating with arbuscular mycorrhiza (AM) might facilitate the herbaceous understory consisting mainly of AM plants.

In a recent study, Veresoglou *et al.* (2017) suggested that AM woody species in a temperate forest facilitate the establishment of AM herbaceous species of the understory. AM woody species might be seen as islands of AM propagules in a large archipelago of non-AMF-associating trees which can support the AMF-associating ones (van der Heijden, 2004). The differences between stands dominated with AMF-associating trees and those dominated with woody plants that do not form AMF associations in Veresoglou *et al.* (2017) were inferred from plant community data and there have been no comparable studies assaying *in situ* AMF availability.

We here addressed this knowledge gap by testing if the abundance of AMF is higher in stands with a higher cover of AMF-associating woody species. For this purpose, we sampled roots of herbaceous plants that were widespread in these stands and assayed the proportion of roots colonized by AMF (term after McGonigle *et al.*, 1990).

The stands were located in unmanaged, continuous, temperate European forests in northwest Germany (53.30°–53.66°N latitude and 9.03°–9.49°E longitude, Supporting Information Table S1). These are described in detail in Wulf (1992) and Naaf & Wulf (2010) and were used in the study of Veresoglou *et al.* (2017). We used vegetation records from Wulf (1992) and identified 13 of the 25 m × 25 m plots (we here described them as stands; Notes S1) so that the canopy of six of them was predominantly ectomycorrhizal-(ECM)-associating (*low* – below 7% relative cover of AMF-associating woody plants) and seven stands with a mostly AMF-associating canopy (*high* – above 49% relative cover of AMF-associating woody plants) (Table S2). Based on the vegetation records we *a priori* identified eight AMF-associating herbaceous plants that were present in the stands (Notes S1): *Ajuga reptans*, *Allium ursinum*, *Brachypodium sylvaticum*, *Circaea lutetiana*, *Geum urbanum*, *Pulmonaria obscura*, *Ranunculus auricomus* and *Sanicula europaea*. Between 28 and 31 May 2017, we assayed roots of representatives of these plant species in as many stands as possible. We excavated the whole root system of the plants to a maximum depth of *c.* 15 cm. In total we obtained 48 root samples from 13 stands to analyse. Six species were found in at least three different stands; however, *B. sylvaticum* and *S. europaea* were found exclusively in stands with a *high* AMF woody cover. We immediately stored plant material in 70% ethanol and kept it at 4°C until further examination. Roots were stained in Trypan blue (Gange *et al.*, 1999) and assessed with the magnified intersection method (McGonigle *et al.*, 1990) (Notes S1).

To address whether arbuscular colonization (i.e. proportion of root length containing arbuscules; McGonigle *et al.*, 1990) was higher in herbaceous plants in the stands with a *high* cover of AMF-associating woody plants (vs low stands) we fitted a linear mixed effects model (Notes S1). We repeated the analysis using per cent hyphal colonization (i.e. proportion of root length containing hyphae) of the roots as response variable (i.e. test whether hyphal colonization was higher in the *high* stands) and we report on this in Notes S2; here we use hyphal colonization to describe exclusively AMF hyphae.

Arbuscular colonization was higher at *high* stands (> 49% AMF-associating woody-cover) compared to *low* (< 7% AMF-associating woody-cover) stands ($F_{1,27} = 6.75$, $P = 0.015$; Fig. 1a; Fig. 1b for a map; Notes S2). Conclusions did not change when we used hyphal colonization as a response variable, instead (Figs S1, S2). Arbuscular colonization can be seen as a proxy of symbiotic exchange, whereas hyphal colonization as a measure of mycorrhizal fungal

biomass (Johnson *et al.*, 2010; Lekberg *et al.*, 2015), comprising two complementary parameters of a mycorrhizal association. Arbuscular and hyphal colonization varied from 0% to 72% and from 0% to 87.5%, respectively (Figs S3, S4; Table S3). Exceptionally in roots of *G. urbanum* we found small differences in arbuscular colonization across the two stand types (23% at *low* and 25% at *high* stands; respective means for hyphal colonization were

28% and 32%; Fig. 1a). The fitted random effect values for stands, presenting proxies of overall AMF abundance per stand, also correlated positively with the relative availability (i.e. cover) of AM woody plants ($r = 0.67$, $P = 0.01$; Fig. 1c).

We here present empirical evidence that AMF root colonization in herbaceous plants relates to AM woody plant coverage in ECM-dominated woody habitats. Our findings complement the analysis

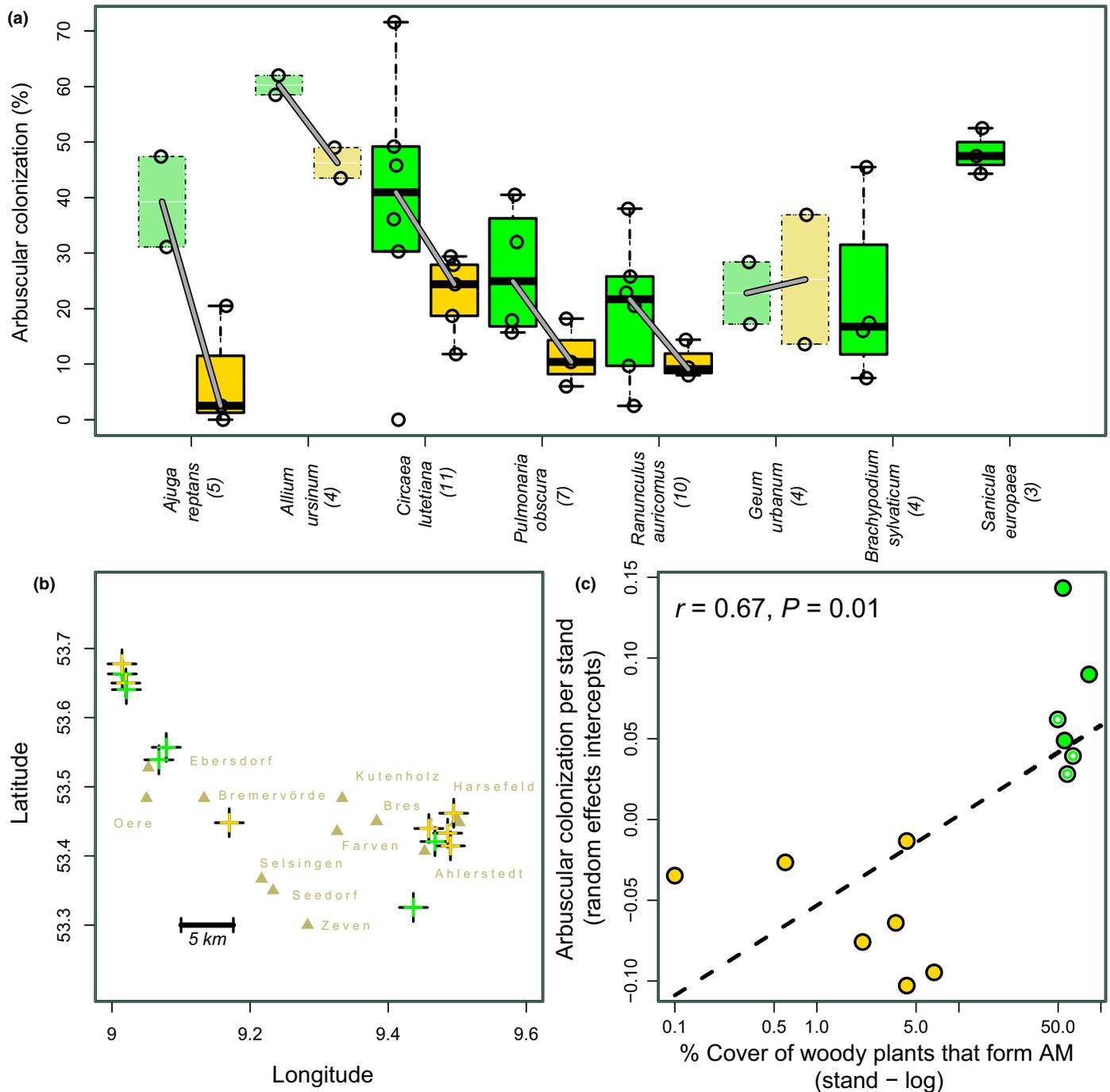


Fig. 1 (a) Arbuscular colonization in the eight target herbaceous plant species (boxplots). Jittered points present the statistics for the sampling stands with *high* (green) or *low* (yellow) coverage of arbuscular mycorrhizal fungi (AMF)-associating woody plant and shrub species. Light coloured boxes originate from only two observations and thus contain higher uncertainties. Model statistics can be found in Supporting Information Notes S2. (b) Map highlighting the geographical position of the stands (green and yellow plus signs between 53.3°–53.7°N latitude and 9.0°–9.5°E longitude) in relation to villages (triangles) in the sampling area. (c) Arbuscular colonization per stand in the form of the respective fitted coefficients (y-axis) plotted against the per cent cover of AMF-associating woody plants and shrubs that were observed in each stand (green dots – *high* and yellow dots – *low*).

of Veresoglou *et al.* (2017) which was carried out in the same study region showing that there were fewer AMF-associating herbaceous plants, and a lower richness of such plant species, in stands with little cover of AMF-associating woody plants. Thereby we support an existing hypothesis that despite their ubiquity, AMF can face dispersal limitation and be present in some habitats at low densities (Zobel & Öpik, 2014).

We presume that the differences in arbuscular colonization have been due to the exclusion of certain AMF taxa from *low* in AMF-associating woody cover stands. We did not assay AMF community structure to test this assumption. Because all plant roots had detectable arbuscular colonization in all stands, it is unlikely that the absence of AMF propagules or nutrient availability-induced changes in host physiology alone explained differences in arbuscular colonization. *Geum urbanum*, for example, responded only weakly to stand type. This might be due to the fact that *G. urbanum* represents the only host plant that is not a forest specialist (Schmidt *et al.*, 2011) and therefore might associate with generalist AMF species, likely with good dispersal properties. Mutualistic networks between AMF and plants are known to be nested and asymmetric (Chagnon *et al.*, 2012). Because of a wider ecological niche, generalist plant species should have the ability to associate with many specialist AMF that could be found in ECM-dominated stands (i.e. habitat generalists come across a wider array of potentially compatible AMF partners) and this might explain the relatively constant colonization in *G. urbanum*. The six other forest specialist species might have a narrower range of AMF associates.

Even though it is woody plants that photosynthesize most in temperate forests, herbaceous plants show the highest species diversity and via contributing unique functional traits to the system, such as palatable leaves high in nitrogen (N) content and fine roots, could be of high ecological importance (Reiss *et al.*, 2009). Herbaceous plants might also interact with the woody canopy through various direct or indirect ways (Gilliam, 2007). As an example, the AM understory could cascade effects to numerous ecosystem processes in soil such as N-mineralization, nitrification and decomposition. We think that the higher litter quality of the AM-understory compared to the other woody plants could be priming these ecosystem processes (Van der Krift & Berendse, 2001; Veresoglou *et al.*, 2011; Luo *et al.*, 2016). The link through AMF between strata presented here implies that islands of AMF-associating trees or shrubs indirectly promote herbaceous plant growth, health and diversity (Azcón-Aguilar & Barea, 1996; van der Heijden *et al.*, 1998) by increasing AMF propagule availability. However, forest management practices are almost exclusively targeting woody species often associating with ECM. In conclusion, mixed stands containing AMF-associating woody plants may effectively promote multi-functionality and multi-diversity in temperate forests.

While root mycorrhizal fungal colonization is also controlled by factors other than propagule availability (including host factors, such as light, or soil factors such as nutrient availability), one interpretation of our results is that herbaceous plants in temperate forests do experience AMF propagule limitation. If this is true, which should be tested in additional work, our study highlights the need to better understand the efficiency of dispersal in Glomeromycotina.

Acknowledgements

The authors acknowledge funding from the Deutsche Forschungsgemeinschaft Project Metacorrhiza (VE 736/2-1) awarded to SDV.

Author contributions

Conceived the study, SDV; carried out the harvest, LG, MW, SDV; assayed root colonization, LG, AM, carried out the statistical analysis, SDV; wrote the article, LG with contributions from SDV; all authors (LG, MW, MCR, AM, SDV) commented on the manuscript and approved the final version.

ORCID

Matthias C. Rillig  <https://orcid.org/0000-0003-3541-7853>
Stavros D. Veresoglou  <https://orcid.org/0000-0001-6387-4109>

**Leonie Grünfeld^{1,2}, Monika Wulf³, Matthias C. Rillig^{1,2} ,
Annette Manntsche¹ and Stavros D. Veresoglou* **

¹Institut für Biologie, Freie Universität Berlin, Altensteinstr. 6,
Berlin D-14195, Germany;

²Berlin-Brandenburg Institute of Advanced Biodiversity Research,
Berlin D-14195, Germany;

³Research Area 2, Leibniz Centre for Agricultural Landscape
Research (ZALF), Eberswalder Straße 84,
Müncheberg D-15374, Germany

(*Author for correspondence: tel +49 30 838 53172;
email sveresoglou@zedat.fu-berlin.de)

References

- Azcón-Aguilar C, Barea J. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—an overview of the mechanisms involved. *Mycorrhiza* 6: 457–464.
- Barbier S, Gosselin F, Balandier P. 2008. Influence of tree species on understory vegetation diversity and mechanisms involved—a critical review for temperate and boreal forests. *Forest Ecology and Management* 254: 1–15.
- Chagnon PL, Bradley RL, Klironomos JN. 2012. Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. *New Phytologist* 194: 307–312.
- Gange AC, Bower E, Stagg PG, Aplin DM, Gillam AE, Bracken M. 1999. A comparison of visualization techniques for recording arbuscular mycorrhizal colonization. *New Phytologist* 142: 123–132.
- Gilliam FS. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience* 57: 845–858.
- van der Heijden MGA. 2004. Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. *Ecology Letters* 7: 293–303.
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglou P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69–72.
- Gould AB, Hendrix JW, Ferriss RS. 1996. Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. I. Propagule and spore population densities. *Canadian Journal of Botany* 74: 247–261.
- Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences, USA* 107: 2093–2098.

- Van der Krift TAJ, Berendse F. 2001. The effect of plant species on soil nitrogen mineralization. *Journal of Ecology* 89: 555–561.
- Lekberg Y, Rosendahl S, Olsson PA. 2015. The fungal perspective of arbuscular mycorrhizal colonization in 'nonmycorrhizal' plants. *New Phytologist* 205: 1399–1403.
- Luo Z, Wang E, Sun OJ. 2016. A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. *Soil Biology and Biochemistry* 101: 96–103.
- Manoharan L, Rosenstock NP, Williams A, Hedlund K. 2017. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. *Applied Soil Ecology* 115: 53–59.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115: 495–501.
- Naaf T, Wulf M. 2010. Habitat specialists and generalists drive homogenization and differentiation of temperate forest plant communities at the regional scale. *Biological Conservation* 143: 848–855.
- Reiss J, Bridle JR, Montoya JM, Woodward G. 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends in Ecology and Evolution* 24: 505–514.
- Schmidt M, Kriebitzsch W, Ewald J. 2011. *Waldartenlisten der Farn- und Blütenpflanzen, Moose und Flechten Deutschlands* (Schmidt M, ed.). Bonn, Germany: BfN.
- Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* 21: 211–220.
- Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A *et al.* 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108: 1028–1046.
- Sutherland WJ, Freckleton RP, Godfray HCJ, Beissinger SR, Benton T, Cameron DD, Carmel Y, Coomes DA, Coulson T, Emmerson MC *et al.* 2013. Identification of 100 fundamental ecological questions. *Journal of Ecology* 101: 58–67.
- Veresoglou SD, Sen R, Mamolos AP, Veresoglou DS. 2011. Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. *Journal of Ecology* 99: 1339–1349.
- Veresoglou SD, Wulf M, Rillig MC. 2017. Facilitation between woody and herbaceous plants that associate with arbuscular mycorrhizal fungi in temperate European forests. *Ecology and Evolution* 7: 1181–1189.
- Wulf M. 1992. Vegetationskundliche und ökologische Untersuchungen zum Vorkommen gefährdeter Pflanzenarten in Feuchtwäldern Nordwestdeutschlands. *Dissertationes Botanicae* 185: 1–246.
- Zobel M, Öpik M. 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities – which drives which? *Journal of Vegetation Science* 25: 1133–1140.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Hyphal colonization R statistics across species.

Fig. S2 Analysis of fitted arbuscular colonization coefficients per stand.

Fig. S3 Variance statistics in root colonization.

Fig. S4 Correlogram of per cent arbuscular colonization.

Notes S1 Extended Materials and Methods.

Notes S2 Detailed statistics.

Table S1 Site information.

Table S2 List of woody species in the study.

Table S3 Raw arbuscular colonization data.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Key words: AMF propagule limitation, arbuscular mycorrhizal fungi (AMF), Glomeromycotina, herbaceous understory, mycorrhizal root colonization, temperate forests.

Received, 16 September 2019; accepted, 27 November 2019.