

# Global imprint of mycorrhizal fungi on whole-plant nutrient economics

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**Mycorrhizal fungi are critical members of the plant microbiome, forming a symbiosis with the roots of most plants on Earth. Most plant species partner with either arbuscular or ectomycorrhizal fungi, and these symbioses are thought to represent plant adaptations to fast and slow soil nutrient cycling rates. This generates a second hypothesis, that arbuscular and ectomycorrhizal plant species traits complement and reinforce these fungal strategies, resulting in nutrient acquisitive vs. conservative plant trait profiles. Here we analyzed 17,764 species level trait observations from 2,940 woody plant species to show that mycorrhizal plants differ systematically in nitrogen and phosphorus economic traits. Differences were clearest in temperate latitudes, where ectomycorrhizal plant species are more nitrogen use- and phosphorus use-conservative than arbuscular mycorrhizal species. This difference is reflected in both aboveground and belowground plant traits and is robust to controlling for evolutionary history, nitrogen fixation ability, deciduousness, latitude, and species climate niche. Furthermore, mycorrhizal effects are large and frequently similar to or greater in magnitude than the influence of plant nitrogen fixation ability or deciduous vs. evergreen leaf habit. Ectomycorrhizal plants are also more nitrogen conservative than arbuscular plants in boreal and tropical ecosystems, although differences in phosphorus use are less apparent outside temperate latitudes. Our findings bolster current theories of ecosystems rooted in mycorrhizal ecology and support the hypothesis that plant mycorrhizal association is linked to the evolution of plant nutrient economic strategies.**

mycorrhizal fungi | plant traits | plant nutrition | nutrient limitation | plant economics

**M**ycorrhizal fungi are critical members of the plant microbiome, enhancing plant access to soil nutrients and water (1). Among mycorrhizal fungi, there are 2 predominant functional types, arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi, which form symbioses with the roots of most plants on Earth (2). A long-standing hypothesis in ecosystem ecology is that AM and EM associations represent contrasting nutrient acquisition strategies (3, 4). Specifically, AM-associated plants rely on inorganic soil nitrogen (N) resources and dominate “fast” N cycling ecosystems with high soil N mineralization rates, while EM-associated plants thrive in “slow” N cycling ecosystems with low soil N mineralization rates, enabled by the ability of EM fungi to degrade and take up organic N from soil (5–8). In support of this idea, anthropogenic inorganic N pollution in North America is associated with a shift in forest composition from EM- to AM-associated tree species (9, 10). While these arguments generally focus on plant N nutrition, in principle this dichotomy may also extend to plant phosphorus (P) acquisition strategies if EM fungi invest more than AM fungi in extracellular P acquisition (8, 11, 12).

Systematic differences in soil nutrient cycling rates between AM and EM ecosystems (13) generate a second hypothesis, that a plant’s mycorrhizal association is correlated with plant nutrient economic traits: specifically, AM fungal-associated plants may be selected to have nutrient acquisitive traits, and EM fungal-associated plants may be selected to have nutrient conservative

traits (3). Such a dichotomy would represent an eco-evolutionary feedback between plants and the soil nutrient environment, mediated by mycorrhizal fungi, selecting for plant traits that reinforce fast or slow nutrient cycling within AM and EM ecosystems.

Despite the hypothesized correlations between mycorrhizal association and plant nutrient economic trait profiles (3), there is conflicting evidence that AM- and EM-associated plant species systematically differ in these traits. Regional and global sampling of thousands of plant species have shown no difference in green foliar N or P concentrations between AM fungal- and EM fungal-associated plants (14, 15). A more recent analysis has shown idiosyncratic variation in foliar nutrient resorption, with AM plants resorbing more N within tropical latitudes and EM plants resorbing more P within boreal latitudes (16). However, plant traits, plant mycorrhizal associations, and plant evolutionary history are not independent (17–19). Given that most studies of EM plants are focused within either the Pinaceae or Fagales lineages (15, 17), it is difficult to determine whether these findings truly emerge due to differences in mycorrhizal habit or whether mycorrhizal association is a proxy for strong correlations between plant traits and plant evolutionary history. The systematic differences observed may reflect traits common among a few plant clades arising independently of plant mycorrhizal association, coupled with nonrandom sampling of plant clades across the plant phylogeny. Understanding whether groups of species differ

## Significance

**Most plants on Earth form a symbiosis with root-associated (i.e., mycorrhizal) fungi, trading plant photosynthate for fungal-acquired soil nutrients. Ecologists have long thought that different types of mycorrhizal fungi—arbuscular vs. ectomycorrhizal—represent adaptations to high vs. low soil nutrient availability. Here we show that these different mycorrhizal associations are linked to differences in a suite of plant traits related to nutrient economic strategies. Ectomycorrhizal plant species are more nutrient use-conservative than arbuscular mycorrhizal plant species, an effect that is robust to controlling for plant growth form and evolutionary history. These findings bolster emerging theories in ecosystem ecology that leverage the ecology of mycorrhizal fungi to better predict ecosystem carbon-nutrient cycle interactions.**

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Data deposition: All code to replicate analyses and figures can be found at [github.com/colinaverill/Averill\\_et\\_al\\_2019\\_myco\\_traits](https://github.com/colinaverill/Averill_et_al_2019_myco_traits).

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systematically in a given trait requires accounting for evolutionary history (20, 21).

Moreover, the analyses reported to date have focused on foliar nutrient concentrations, although differences between AM and EM plant nutritional strategies may be more related to below-ground traits than to aboveground traits, consistent with slower root litter decomposition of EM compared with AM plant species (22, 23). Plants can invest as much or more carbon in fine roots as in aboveground foliage (24), and systematic differences in plant nutrient economic traits may manifest in root nutrient concentrations rather than in green or senescent foliar nutrient concentrations.

Finally, in addition to nutritional strategy differences between mycorrhizal types, biogeochemical theory predicts that plants shift from N to P limitation from the poles to the tropics (25). Plants within high-latitude boreal-arctic ecosystems on younger soils are predicted to be primarily N-limited, while low-latitude tropical forests on old, highly weathered soils are predicted to be P-limited (25, 26). If EM plants are inherently more nutrient use-conservative than AM plants, and if the primary element limiting plant growth shifts from N to P from the poles to the tropics, then we may predict EM plants to be more N use-conservative compared with AM plants at high latitudes and more P use-conservative at low latitudes. Alternatively, if N and P strategies are fundamentally linked to an overall plant nutritional strategy, then we predict EM plants to be more N and P use-conservative than AM plants across all latitudes.

Here we analyzed 17,764 species-level observations of mycorrhizal association, N fixation status, plant growth form, deciduous vs. evergreen leaf habit, green foliage, senescent foliage, and root element concentrations across 2,940 woody plant species (Fig. 1 and *SI Appendix, Table S1*). First, we used these data to test the hypothesis that AM- and EM-associated plants differ systematically in plant nutrient economic traits and to determine whether this effect is independent of climate, plant growth form, N fixation ability, deciduous vs. evergreen leaf habit, and evolutionary history by including all these predictors in a single statistical model. Second, we tested for interactions between mycorrhizal associations and latitudinal zone (boreal, temperate, tropical), to understand how correlations between mycorrhizal association and nutrient use may change across latitudes. Because data coverage is heterogeneous across mycorrhizal associations and latitudinal zones, we assessed our ability to detect significant differences in plant nutrient economic traits between mycorrhizal associations, given our sample size and phylogenetic autocorrelation

structure, by comparing posterior distributions of mycorrhizal trait estimates relative to biologically meaningful effect sizes.

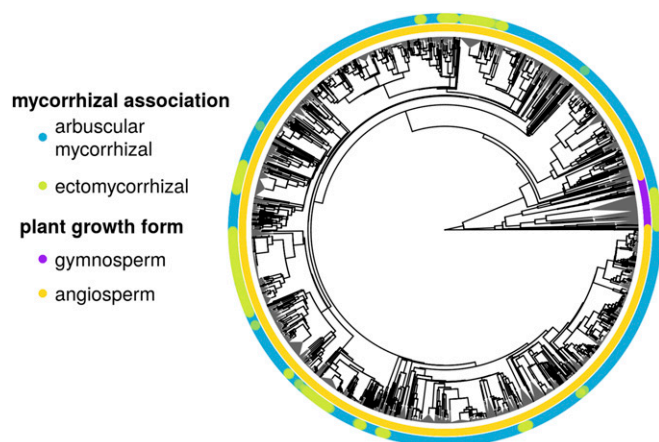
## Results

EM plant species traits were systematically more nutrient use-conservative across most traits measured, and the effects were clearest for N traits across all latitudes. EM plants also had more conservative P traits in temperate latitudes, where data were most abundant (Fig. 2). Green foliar N correlations with mycorrhizal association were strong and independent of latitudinal zone. EM green foliar N values were ~10–14% lower than AM values in all latitudinal zones, although the difference was significant only in temperate and tropical latitudes (Bayesian  $P$  values <0.05; Fig. 2A). We emphasize that even when latitudinal zone main effects and interactions are not significant, they can still drive insignificant AM-EM contrasts within latitudinal zones, as these terms also propagate uncertainty associated with differences in sample sizes across latitudinal zones. This generally occurs when a small sample size within a latitudinal zone drives large latitudinal parameter uncertainty, which propagates through to latitude-specific mycorrhizal trait estimates. Senescent foliar N values varied by latitudinal zone and mycorrhizal type. Senescent foliar N values were 35% to 48% lower in EM plants compared to AM plants within boreal and temperate latitudes (Bayesian  $P$  values <0.05) but were similar between mycorrhizal associations within tropical latitudes (Fig. 2B). Root N values differed by mycorrhizal association independent of latitudinal zone, with EM plants having 19% to 32% less N in roots compared to AM plants across all latitudes (Bayesian  $P$  values <0.05; Fig. 2C).

Differences in P traits between mycorrhizal types were more complex than differences in N traits. Green foliar P concentrations were affected by mycorrhizal association and latitudinal zone (Bayesian  $P$  value <0.05). Green foliar P concentrations were 18% to 26% lower in EM plants compared with AM plants in temperate and boreal latitudes (Fig. 2D) and ~8% lower in tropical latitudes, although the difference within tropical latitudes was not significant (Bayesian  $P$  value >0.05). Senescent foliar P responses were also affected by latitudinal zone but were more variable, likely due to smaller sample size. Differences in senescent foliar P between AM and EM mycorrhizal types were never significant (Fig. 2E). Root P concentrations were dependent on latitudinal zone. Mycorrhizal fungal association was an important predictor in models of root P concentrations; however, posterior contrasts showed a significant difference between plant mycorrhizal associations only within temperate latitudes, where EM root P concentrations were ~32% lower than AM root P concentrations (Bayesian  $P$  value = 0.03; Fig. 2F). EM root P concentrations were 19% and 32% lower in tropical and boreal latitudes, respectively; however, these differences were not significant.

We found that phylogeny alone could explain a substantial amount of trait variation for several traits. Analysis of plant traits as a function of phylogenetic distance alone (without environmental and ecological covariates) showed a substantial phylogenetic signal in plant traits (Pagel's  $\lambda$  = 0.40 to 0.81). Phylogeny alone explained 15% to 38% of the variation in plant trait values, depending on the trait (Fig. 3). When we visualized AM-EM contrasts for all traits within temperate latitudes before and after accounting for phylogeny (*SI Appendix, Fig. S2*), the magnitude of trait values between mycorrhizal associations often changed substantially, although qualitative differences between AM and EM plant traits did not change. Incorporating phylogeny generally increased parameter uncertainty, as phylogenetic residual autocorrelation reduced statistical power. However, this increased parameter uncertainty was not large enough to mask significant differences between EM and AM plants in nutrient economic traits within temperate latitudes.

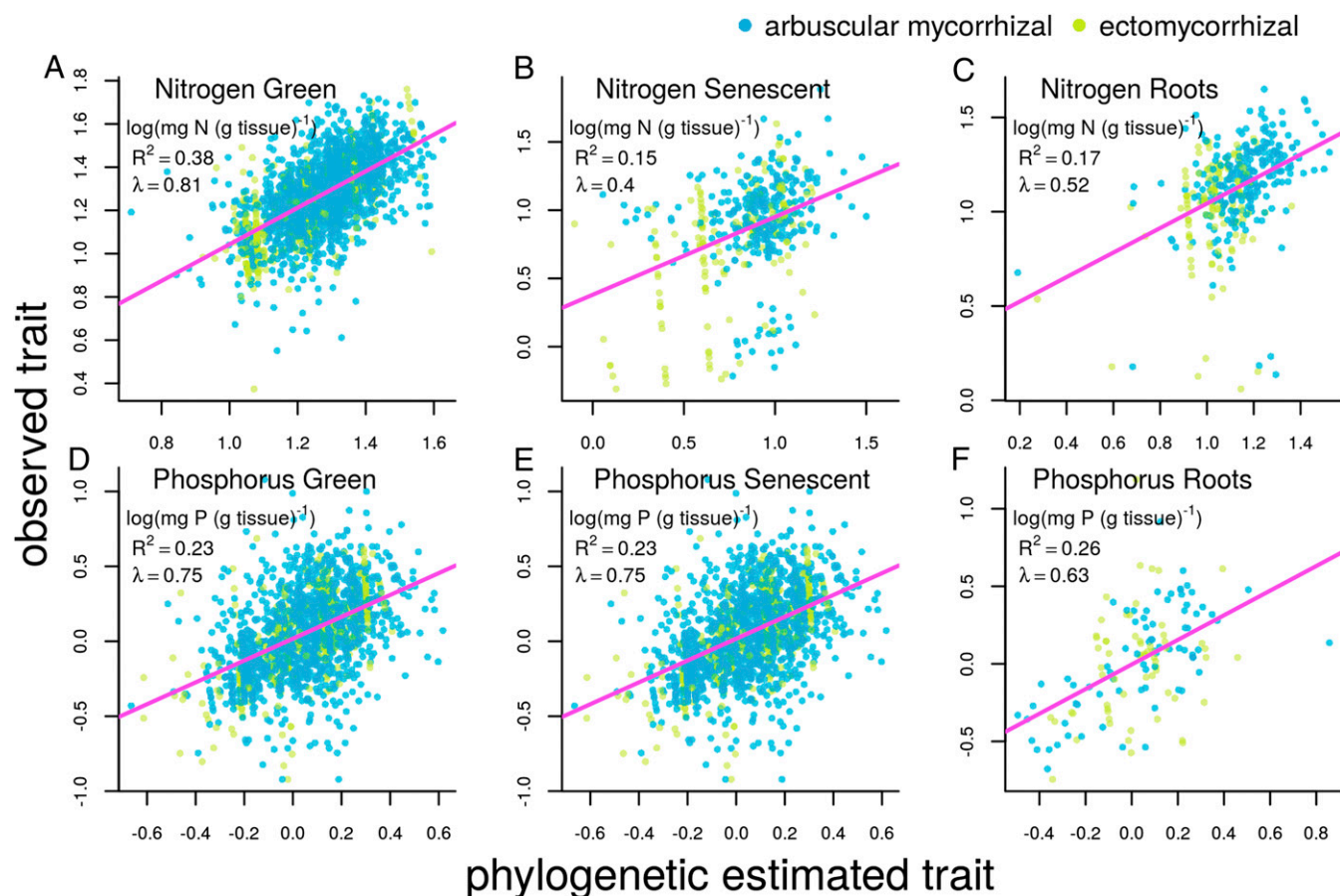
N-fixing plant species had greater green foliar N ( $P$  = 0.01), senescent foliar N ( $P$  = 0.008), and root N ( $P$  = 0.026)



**Fig. 1.** Phylogeny of all plant species used in this analysis. The inner ring identifies which species are angiosperms (orange) vs. gymnosperms (purple); the outer ring, which species are arbuscular (blue) vs. ectomycorrhizal (green).







**Fig. 3.** Phylogenetic estimated vs. observed plant trait values: N green (A), N senescent (B), N roots (C), P green (D), P senescent (E), P roots (F). All values are log10-transformed. Estimated trait values were generated based on phylogenetic models fit to all data except for the plant species being estimated or leave-one-out cross-validation. Dashed lines indicate nonsignificant relationships between predicted vs. observed values.

EM plant species had significantly lower green foliar N concentrations than AM plant species. This finding is in contrast with the results of previous comparative studies, which detected no difference between mycorrhizal associations, possibly due to a difference in statistical power among analyses. For instance, an analysis of United Kingdom plant N and P concentrations compared 12 EM species and 53 AM species (14) but was unable to definitively identify the influences of mycorrhizal status and deciduousness. A second study examined many more species within a phylogenetic framework, but a lack of phylogenetic resolution necessitated the use of a statistical technique that reduced the dataset to only 17 paired-clade comparisons (15). That test has the advantage of being conservative, but also the disadvantage of reducing statistical power (30) because it cannot make use of all of the species-level data in a single test. Our analysis uses a phylogenetic framework to take information from each species-level observation, increasing the statistical power to more definitively distinguish differences between EM and AM plants hinted at in previous studies. A large phylogenetic analysis of root N concentrations found no difference between AM vs. EM plants, yet most AM observations were nonwoody, potentially reflecting an undersampling of woody plants (31).

It is important to recognize that many previous analyses comparing AM and EM traits do not account for phylogenetic autocorrelation, especially at the site scale. Indeed, our findings are consistent with previous site-scale studies within geographic regions that found differences in AM and EM nutrient economic traits (32, 33). While controlling for phylogeny may affect parameter estimates substantially, our analysis suggests that previous

conclusions based on AM vs. EM contrasts may be valid; nonetheless, we emphasize that inference may be biased in studies without very large sample sizes (*SI Appendix, Fig. S2*). Furthermore, the goal of the present analysis was to address a question that could be answered only by considering many plant species across the plant phylogeny. Our findings should not be interpreted to suggest that other conclusions based on site-scale studies of fewer species do not have merit.

Green foliar N and root N patterns were similar across latitudes, while past analyses have provided mixed support for coordinated foliar and root nutrient economic strategies (34). Our analysis suggests that some of this discrepancy may be due to a lack of accounting for differences in plant mycorrhizal association in previous analyses, although raw correlations between species level green foliar N and root N are weak in this dataset ( $R^2 = 0.04$ ;  $P < 0.001$ ). It may be that this coordination can be seen only after species are binned into functional groups, as done in this analysis. Future work on aboveground-belowground trait coordination should explore the dominant form of mycorrhizal association as a potential driver, as well as the many other known controls over foliar and root nutrient concentrations described here and in many other analyses (34).

We observed remarkable intraspecific trait variation across traits. Approximately 60% of the variation in senescent foliar N and root N could be attributed to intraspecific variation, although we note that our approach will also combine parameter and observation uncertainty into the estimation of intraspecific variation. Other work has shown that intraspecific trait relationships can substantially deviate from interspecific plant economic relationships



12. D. J. Read, J. Perez-Moreno, Mycorrhizas and nutrient cycling in ecosystems—A journey towards relevance? *New Phytol.* **157**, 475–492 (2003).
13. G. Lin, M. L. McCormack, C. Ma, D. Guo, Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytol.* **213**, 1440–1451 (2017).
14. J. Cornelissen, R. Aerts, B. Cerabolini, M. Werger, M. van der Heijden, Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia* **129**, 611–619 (2001).
15. N. Koele, I. A. Dickie, J. Oleksyn, S. J. Richardson, P. B. Reich, No globally consistent effect of ectomycorrhizal status on foliar traits. *New Phytol.* **196**, 845–852 (2012).
16. H.-Y. Zhang *et al.*, Foliar nutrient resorption differs between arbuscular mycorrhizal and ectomycorrhizal trees at local and global scales. *Glob. Ecol. Biogeogr.* **27**, 875–885 (2018).
17. I. A. Dickie, B. Moyersoen, Towards a global view of ectomycorrhizal ecology. *New Phytol.* **180**, 263–265 (2008).
18. N. G. Swenson, Phylogenetic imputation of plant functional trait databases. *Ecography* **37**, 105–110 (2014).
19. N. Wurzburger, E. N. J. Brookshire, M. L. McCormack, R. A. Lankau, Mycorrhizal fungi as drivers and modulators of terrestrial ecosystem processes. *New Phytol.* **213**, 996–999 (2017).
20. J. Felsenstein, Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).
21. R. P. Freckleton, P. H. Harvey, M. Pagel, Phylogenetic analysis and comparative data: A test and review of evidence. *Am. Nat.* **160**, 712–726 (2002).
22. C. R. See *et al.*, Global patterns in fine root decomposition: Climate, chemistry, mycorrhizal association and woodiness. *Ecol. Lett.* **22**, 946–953 (2019).
23. T. Sun *et al.*, Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 10392–10397 (2018).
24. K. E. Clemmensen *et al.*, Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**, 1615–1618 (2013).
25. P. B. Reich, J. Oleksyn, Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11001–11006 (2004).
26. P. M. Vitousek, R. W. Howarth, Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* **13**, 87–115 (1991).
27. M. Shi, J. B. Fisher, E. R. Brzostek, R. P. Phillips, Carbon cost of plant nitrogen acquisition: Global carbon cycle impact from an improved plant nitrogen cycle in the Community Land Model. *Glob. Change Biol.* **22**, 1299–1314 (2016).
28. E. R. Brzostek, K. T. Rebel, K. R. Smith, R. P. Phillips, “Integrating mycorrhizas into global scale models” in *Mycorrhizal Mediation of Soil*, N. C. Johnson, C. Ghering, J. Jansa, Eds (Elsevier, 2017), pp. 479–499.
29. C. Terrer *et al.*, Ecosystem responses to elevated CO<sub>2</sub> governed by plant-soil interactions and the cost of nitrogen acquisition. *New Phytol.* **217**, 507–522 (2018).
30. D. D. Ackerly, Taxon sampling, correlated evolution, and independent contrasts. *Evolution* **54**, 1480–1492 (2000).
31. O. J. Valverde-Barrantes, G. T. Freschet, C. Roumet, C. B. Blackwood, A worldview of root traits: The influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. *New Phytol.* **215**, 1562–1573 (2017).
32. L. H. Comas, D. M. Eissenstat, Patterns in root trait variation among 25 co-existing North American forest species. *New Phytol.* **182**, 919–928 (2009).
33. L. H. Comas, H. S. Callahan, P. E. Midford, Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: Implications for the evolution of belowground strategies. *Ecol. Evol.* **4**, 2979–2990 (2014).
34. P. B. Reich, The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *J. Ecol.* **102**, 275–301 (2014).
35. L. D. L. Anderegg *et al.*, Within-species patterns challenge our understanding of the leaf economics spectrum. *Ecol. Lett.* **21**, 734–744 (2018).
36. S. D. Torti, P. D. Coley, T. A. Kursar, Causes and consequences of monodominance in tropical lowland forests. *Am. Nat.* **157**, 141–153 (2001).
37. A. Kohler *et al.*; Mycorrhizal Genomics Initiative Consortium, Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet.* **47**, 410–415 (2015).
38. I. J. Wright *et al.*, The worldwide leaf economics spectrum. *Nature* **428**, 821–827 (2004).
39. B. S. Steidinger *et al.*; GFB consortium, Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* **569**, 404–408 (2019).
40. R. P. Freckleton, N. Cooper, W. Jetz, Comparative methods as a statistical fix: The dangers of ignoring an evolutionary model. *Am. Nat.* **178**, E10–E17 (2011).