

Running head: Reconciling lignin decomposition models

Lignin lags, leads, or limits the decomposition of litter and soil organic carbon

Steven J. Hall^{1*}, Wenjuan Huang¹, Vitaliy I. Timokhin², Kenneth E. Hammel^{3,4}

¹Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, United States

²University of Wisconsin, Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, Madison, Wisconsin 53706, United States

³US Forest Products Laboratory, Madison, WI 53726, United States

⁴Department of Bacteriology, University of Wisconsin, Madison, WI 53706, United States

*Corresponding author

Email: stevenjh@iastate.edu; Phone: 515-294-7650; Fax: 515-294-1337

24 **Abstract**

25 Lignin's role in litter and soil organic carbon (SOC) decomposition remains contentious. Lignin
 26 decomposition was traditionally thought to increase during mid-stage litter decomposition, when
 27 cellulose occlusion by lignin began to limit mass loss. Alternatively, lignin decomposition could
 28 be greatest in fresh litter as a consequence of co-metabolism, and lignin might decompose faster
 29 than bulk SOC. To test these competing hypotheses, we incubated 10 forest soils with C₄ grass
 30 litter (amended with ¹³C-labeled or unlabeled lignin) over two years and measured soil
 31 respiration and its isotope composition. Early lignin decomposition was greatest in five of ten
 32 soils, consistent with the co-metabolism hypothesis. However, lignin decomposition peaked 6–
 33 24 months later in the other five soils, consistent with the substrate-limitation hypothesis; these
 34 soils were highly acidic. Rates of lignin, litter, and SOC decomposition tended to converge over
 35 time. Cumulative lignin decomposition was never greater than SOC decomposition; lignin
 36 decomposition was significantly lower than SOC decomposition in six soils. Net nitrogen
 37 mineralization predicted lignin decomposition ratios relative to litter and SOC. Although the
 38 onset of lignin decomposition can indeed be rapid, lignin still presents a likely bottleneck in litter
 39 and SOC decomposition, meriting a reconsideration of lignin's role in modern decomposition
 40 paradigms.

41 *Key words: carbon stable isotopes, lignin, litter decomposition, litter quality, nitrogen*
 42 *mineralization, soil organic matter*

43 **Introduction**

44 Lignin once had a primary role in conceptual and mechanistic models of litter and soil
 45 organic carbon (SOC) decomposition, but its importance in controlling these fluxes has been
 46 increasingly challenged. Lignin is a ubiquitous constituent of higher plant tissue that is

characterized by a structurally diverse assemblage of phenylpropane units that form protective associations with carbohydrates. Polymeric lignin can only be cleaved by reaction with strongly oxidizing chemical species—typically produced by specialized enzymes and/or the biologically mediated redox cycling of metals (Kirk and Farrell 1987, Hammel et al. 2002). Lignin decomposition, therefore, was long thought to limit overall rates of litter mass loss after unprotected carbohydrates had been consumed (Berg and Staaf 1980, Melillo et al. 1982, Adair et al. 2008, Talbot and Treseder 2012). Lignin content is included as an influential parameter in mechanistic C cycling models such as Century (Parton et al. 1987). Ultimately, lignin decomposition products were thought to accumulate in soil, representing a major portion of SOC (Waksman and Iyer 1932, Bollag et al. 1997).

Recent work has challenged these views on several fronts. Lignin might decompose fastest during early decomposition as a consequence of co-metabolic degradation with labile C substrates—an argument that carbohydrate availability drives lignin decomposition, not vice-versa as had been previously assumed (Klotzbücher et al. 2011). Separate research challenged the significance of lignin-derived C in SOC. Analyses of extractable lignin phenols suggested that lignin might decompose faster than SOC as a whole (Amelung et al. 2008, Thevenot et al. 2010), and spectroscopic analyses indicated a relatively low abundance of lignin derivatives in SOC in some soils (Kögel-Knabner et al. 2008, Grandy and Neff 2008). These findings are consistent with arguments based on microbial substrate-use-efficiency. Because a minor fraction of lignin-derived C is typically assimilated in microbial biomass (Martin et al. 1980), we might expect C in efficiently assimilated compounds such as carbohydrates and proteins to persist in soil longer than lignin-derived C by repeatedly cycling through living and dead microbial biomass (Cotrufo et al. 2013).

However, methodological concerns have long plagued examinations of lignin dynamics (Crawford 1981, Kirk and Farrell 1987, Klotzbücher et al. 2016). Perhaps most importantly, measurements of cupric-oxide extractable lignin phenols which underpinned recent reports of rapid lignin decomposition can greatly underestimate soil lignin content (Hernes et al. 2013). Indeed, recent work demonstrated enrichment of lignin-derived C in mineral-associated SOC, contrasting with previous reports (Huang et al. 2019). Here, we reevaluated predictions of classical and modern conceptual models of lignin decomposition by quantifying lignin, litter, and SOC decomposition over time. We employed a stable isotope approach following the seminal work of Haider and colleagues (Martin et al. 1980, Haider and Martin 1981), preparing high-molecular-weight lignin with a ^{13}C label at the C_β position of the propyl sidechain (Hall et al. 2015). Release of lignin C_β as carbon dioxide (CO_2) provides unequivocal evidence that the lignin polymer was cleaved (Kirk and Farrell 1987, Kirk and Brunow 1988). Parallel samples received lignin without an isotope label to quantify decomposition of litter C from a C_4 grass and from extant SOC using natural abundance stable isotopes (Hall et al. 2017).

Diverse ecological factors, especially nitrogen (N) availability, may influence decomposition of litter and lignin. Whereas greater N availability tends to increase early decomposition rates, it may decrease late-term decomposition by suppressing enzymes that mediate lignin breakdown (Craine et al. 2007, Chen et al. 2018). Therefore, variation in N availability could provide critical context for evaluating competing conceptual models (Fig. 1) of lignin decomposition vis-à-vis total litter and SOC. We incubated 10 forest soils that spanned broad N availability gradients (Appendix 1: Table S1). We tested the following alternative hypotheses: first, lignin decomposes fastest during early decomposition in conjunction with high litter decomposition rates (co-metabolism hypothesis). Alternatively, lignin decomposition

increases after labile substrates have been depleted, at which point lignin decomposition limits overall litter decomposition (substrate limitation hypothesis). Second, lignin decomposes faster than bulk SOC, as would be predicted from microbial substrate-use efficiency. Alternatively, lignin decomposes at similar or slower rates as bulk SOC, consistent with oxidative depolymerization as a rate-limiting step of decomposition.

Materials and Methods

We collected surface mineral soils (0–10 cm, surface litter removed) from 10 North American forests spanning a broad range of edaphic characteristics (Appendix 1: Table S1). Samples were stored field-moist in polyethylene bags at 4° C for several weeks prior to the incubation. Standing senesced above-ground stem and leaf tissue (litter) from *Andropogon gerardii* Vitman, a C₄ grass, was collected near Ames, IA, USA (42.04 °N, -93.60 °W), dried at 65 °C, and ground to pass a 250 µm mesh. The litter had 41.3% C and 1.1% N by mass (C:N ratio of 37.5) and a δ¹³C value of -13.1‰.

¹³C-labeled and unlabeled lignins We prepared synthetic guaiacyl lignin following Kirk & Brunow (1988) which contained either a ¹³C label (99 atom%) or natural abundance C at the C_β position of each C₉ substructure. Lignins were fractionated by gel permeation chromatography to obtain a high-molecular-weight fraction (> 1000 Da). The average labeled phenylpropane subunit has a molecular mass of 197, so the ¹³C label represented 13/197 of total lignin mass. Lignins were precipitated on the *A. gerardii* litter in a 1:21 mass ratio by dissolution in a 1:4 solution of acetone and water and evaporation in a fume hood. The lignin-litter mixture had a C:N ratio of 38.3. Further preparation and characterization details are given in Hall et al. (2015).

Soil incubation experiments Each soil was gently homogenized (not sieved) after coarse roots and rocks were removed, and replicate subsamples (1 g dry mass equivalent) were used for

incubations. Two treatments were imposed with three replicates for each soil: amendment with 100 mg *A. gerardii* litter + natural abundance lignin, or with 100 mg *A. gerardii* litter + $^{13}\text{C}_\beta$ -labeled lignin. This litter:soil ratio is representative of moderate litter inputs to surface soil (1 kg m^{-2}). Samples were incubated in 50 ml centrifuge tubes placed inside 946-ml glass jars. Deionized water was added as needed to achieve field moisture capacity, determined for each soil by saturating and draining a subsample through filter paper. Jars were sealed with Viton gaskets and stainless steel lids with butyl rubber septa for headspace sampling/flushing. Jars were vented to the atmosphere with a needle and purged with humidified CO_2 -free air. Accumulated CO_2 was periodically sampled using a syringe with needle and stopcock and jars were again purged with CO_2 -free air. Sampling intervals increased from weekly to biweekly to monthly to bimonthly over the two-year incubation (Fig. 2) to ensure sufficient CO_2 accumulation for precise analysis. Concentrations of CO_2 were always <5000 ppm, indicating that oxygen limitation did not occur (assuming an approximately equimolar ratio of headspace oxygen consumed to CO_2 produced). We thus measured the entire cumulative production of CO_2 from each sample. Mole fractions and $\delta^{13}\text{C}$ values of CO_2 were analyzed with a tunable diode laser (TGA200A, Campbell Scientific, Logan UT, USA; Hall et al., 2017). The long-term mean precision of $\delta^{13}\text{C}$ was 0.33‰ (1 SD) and the relative SD of CO_2 mole fractions was 1.1%. Water was added gravimetrically at 2–4-month intervals to replenish vapor lost during headspace flushing.

Data analysis We calculated CO_2 production from bulk litter, lignin C_β , and SOC using isotope mixing models (Appendix 1: Table S1). We assessed temporal trends in C losses using generalized additive mixed models (GAMMs) fit to each C source in each soil, including an autoregressive error term to account for temporal autocorrelation, using the mgcv package

(Wood 2017) version 1.8.28 in R version 3.6.0. Differences in total decomposition among C pools within a given soil were tested with ANOVA and Tukey's HSD. We used vector analysis of lignin:litter and lignin:SOC decomposition ratios (Moorhead et al. 2016; Appendix 1: Figure S2) to test the tripartite relationships of lignin, litter, and SOC decomposition with predictor variables (Appendix 1: Table S1).

Results

Decomposition of litter, lignin, and SOC By the end of the experiment, 43–57% of litter C had decomposed among soils, whereas only 5–12% of lignin C_β and 12–37% of SOC had decomposed (Appendix 1: Table S2, Fig. S1). Total lignin C_β decomposition was unrelated to total litter or SOC decomposition among soils ($R^2 = 0.02$ and $R^2 = 0.16$, respectively; $P > 0.05$). Litter decomposition rates were greatest early in the experiment and declined thereafter, punctuated by temporary plateaus (Fig. 2). In contrast, lignin decomposition rates significantly increased over time relative to their initial values in at least five of the soils (upper panels in Fig. 2), as indicated by the GAMMs. In these soils, lignin decomposition rates peaked many months later (days 147–308) or were still increasing at the end of the two-year experiment. In the other five soils (lower panels in Fig. 2), lignin decomposition rates were greatest within the first two weeks of the experiment and subsequently declined. The soils where lignin decomposition rates increased relative to initial values were strongly acidic (pH < 4.9; Appendix 1: Table S1), whereas the other soils had weakly acidic to neutral pH values (5.7–7.2). Net N mineralization ranged from negative to positive values among soils and increased with total litter C decomposition ($R^2 = 0.65$, $P = 0.005$). Total lignin decomposition was unrelated to N mineralization, whether measured in samples with or without added litter ($P > 0.05$). However, expressing ratios of lignin:litter and lignin:SOC decomposition as vectors (Appendix 1: Fig. S2b)

showed that N mineralization (measured in litter-free samples) increased in soils where lignin decomposition was greater relative to SOC decomposition than litter decomposition ($P = 0.03$). The overall magnitude of lignin decomposition vs. litter and SOC also increased with N mineralization ($P = 0.02$; Appendix 1: Fig. S2c).

Pairwise relationships between decomposition fluxes Relative decomposition rates (expressed as fractional losses of the initial C pools) were initially greater for litter C than lignin C_β by > 1 –2 orders of magnitude and remained higher throughout the experiment (Fig. 2). However, litter and lignin C_β decomposition rates within individual soils moved increasingly closer to a 1:1 relationship over time, albeit at different rates (Fig. 3). In the five soils where lignin C_β decomposition increased over time relative to initial values, the period of increase was associated with an approach to a 1:1 relationship with litter C decomposition rates, which was maintained throughout the rest of the experiment (decomposition rates moved approximately parallel to the 1:1 line). In the other five soils, decomposition rates gradually approached the 1:1 line over time. The GAMMs for at least two of these soils indicated that lignin C_β decomposition rates were increasing at the end of the experiment (Fig. 2; CA_Dystroxerept and UT_Haplocryalf).

Similar to their relationships with litter decomposition, lignin C_β decomposition rates were almost always lower than SOC decomposition and tended to converge towards the 1:1 line as the experiment proceeded (Fig. 4). Most soils also showed consistent trends in lignin:litter and lignin:soil decomposition ratios which tracked along or approached a 1:1 relationship over time (Appendix 1: Fig. S3). At the end of the experiment, decomposition of lignin C_β (4–8%) did not differ significantly from SOC decomposition (12–13%) in four soils; the other six soils had lower decomposition of lignin (5–12%) than SOC (16–37%; Appendix 1: Table S2).

Discussion

Our findings reconcile aspects of competing conceptual models that have been used to predict relationships among the decomposition of lignin, litter, and SOC. We found partial support for both alternatives of our first hypothesis: in half of the soils examined, lignin decomposition was greatest early in the experiment and generally decreased thereafter, consistent with the hypothesis that increased labile C supply drives co-metabolic decomposition of lignin during early decomposition (Klotzbücher et al. 2011). However, in the other five soils, lignin decomposition subsequently increased and exceeded initial values after many months. This is consistent with the substrate limitation hypothesis, whereby lignin decomposition increases as carbohydrate availability decreases to enable access to protected substrates (Berg and Staaf 1980). Even across these contrasting groups of soils, however, we found common threads of evidence supporting both co-metabolism and substrate limitation.

The predominance of different drivers of lignin decomposition during different portions of the litter decomposition sequence might explain the overall decoupling of total lignin and litter decomposition across soils. For example, the soils that showed lagged increases in lignin decomposition after months–years also exhibited relatively high rates early in the experiment, consistent with a role for labile C in stimulating co-metabolic lignin degradation. From the opposite perspective, some of the soils that showed overall declining rates of lignin decomposition also showed transient increases at various points in time, consistent with upregulation of lignin decomposition in response to substrate limitation. Finally, all soils eventually trended towards a 1:1 relationship between decomposition rates of lignin and litter C, consistent with the ultimate establishment of lignin depolymerization as the rate-limiting step of litter decomposition. This provides a direct corollary for a long-standing observation that litter lignin content is an effective predictor of late-term decomposition rates (Melillo et al. 1982,

Adair et al. 2008) and challenges the notion that lignin decomposition rates decline disproportionately over time relative to total litter decomposition. Furthermore, initial lignin decomposition rates were unrelated to cumulative lignin decomposition, also consistent with the importance of progressive substrate limitation in eventually promoting lignin breakdown.

What ecological factors, then, might predict differences in the temporal patterns of lignin decomposition rates and cumulative lignin losses among soils? Availability of N might play an important role, as N enrichment has been linked to suppression of lignin decomposition and accumulation of SOC at local and global scales (Chen et al. 2018, Zak et al. 2019). Here, we did not observe a direct relationship between cumulative lignin decomposition and any metric of N availability (C:N ratio, initial mineral N concentrations, or net N mineralization in litter-amended samples or controls). However, soils which had greater decomposition of lignin relative to other C sources, especially SOC, had greater N mineralization (when measured without litter amendment). We cannot ascertain here whether increased N mineralization was a cause or consequence of greater relative lignin decomposition.

We also found circumstantial evidence for the role of soil pH as a control on lignin-degrading organisms. The soils which exhibited lagged increases in lignin decomposition rates had strongly acidic pH values, which often increase fungal growth and diversity relative to bacteria (Rousk et al. 2009, Tedersoo et al. 2014). Although some bacteria may play a role, filamentous fungi likely dominate lignin degradation (Ruiz-Dueñas and Martínez 2009, Bugg et al. 2011). Therefore, increased fungal growth relative to bacteria in a low-pH environment may have enabled the lagged increase in lignin decomposition rates over time in the highly acidic soils. In contrast, competition with bacteria for C or nutrients may have suppressed additional

growth of lignin-degrading fungi in soils with higher pH (Rousk et al., 2009), contributing to decreasing lignin decomposition rates over time.

Decomposition of lignin vs. SOC Consistent with the alternative to our second hypothesis, we found that total lignin decomposition was always equivalent to or lower than total SOC decomposition, contrasting with observations (based on cupric oxide phenol extraction) that lignin decomposed faster than bulk SOC (Amelung et al. 2008, Thevenot et al. 2010). However, these previous conclusions should be viewed with caution given that lignin phenol extractions are non-quantitative in mineral soils (Hernes et al. 2013). In our study, we found that instantaneous and cumulative relationships between lignin and SOC decomposition varied among soils, but that lignin and SOC decomposition rates tended to converge over time. This relationship may reflect an overall rate-limiting importance of oxidative depolymerization reactions, among other factors, in controlling SOC decomposition in these soils.

Overall, our data suggest a partial reconciliation of older and newer ideas surrounding the role of lignin in SOC. In contrast to recent proposals (Thevenot et al. 2010, Klotzbücher et al. 2011, Cotrufo et al. 2013), lignin decomposition often lagged or limited, not led, the decomposition of litter, and total lignin decomposition was similar or lower than that of SOC. Acknowledging that lignin derivatives do not persist indefinitely simply due to their molecular structure (Kleber 2010), a richer understanding of the rates and controls on lignin decomposition may yet prove insightful for predicting SOC persistence—a classic ecological idea (Waksman and Iyer 1932) that has recently fallen out of favor.

Acknowledgements

The soil incubation data are freely available from the Environmental Data Initiative data portal: <https://doi.org/10.6073/pasta/ca93711d9b792e424a74457345db1c24>. We thank an anonymous

reviewer for suggesting use of vector analysis and A. Mirabito for lab assistance. Funding was provided by NSF DEB-1802745 and EAR-1331841. Lignin production was partially funded by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494, BER DE-SC0012742).

Literature Cited

- Adair, E. C., W. J. Parton, S. J. Del Grosso, W. L. Silver, M. E. Harmon, S. A. Hall, I. C. Burke, and S. C. Hart. 2008. Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. *Global Change Biology* 14:2636–2660.
- Amelung, W., S. Brodowski, A. Sandhage-Hofmann, and R. Bol. 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. *Advances in Agronomy* 100:155–250.
- Berg, B., and H. Staaf. 1980. Decomposition rate and chemical changes of scots pine needle litter. II. Influence of chemical composition. *Ecological Bulletins* 32:373–390.
- Bollag, J.-M., J. Dec, and P. M. Huang. 1997. Formation mechanisms of complex organic structures in soil habitats. Pages 237–266 *in* Donald L. Sparks, editor. *Advances in Agronomy*. Academic Press.
- Bugg, T. D., M. Ahmad, E. M. Hardiman, and R. Singh. 2011. The emerging role for bacteria in lignin degradation and bio-product formation. *Current Opinion in Biotechnology* 22:394–400.
- Chen, J., Y. Luo, K. J. van Groenigen, B. A. Hungate, J. Cao, X. Zhou, and R. Wang. 2018. A keystone microbial enzyme for nitrogen control of soil carbon storage. *Science Advances* 4:eaq1689.

- 275 Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial
 276 Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition
 277 with soil organic matter stabilization: do labile plant inputs form stable soil organic
 278 matter? *Global Change Biology* 19:988–995.
- 279 Craine, J., C. Morrow, and N. Fierer. 2007. Microbial nitrogen limitation increases
 280 decomposition. *Ecology* 88:2105–2113.
- 281 Crawford, R. L. 1981. Lignin biodegradation and transformation. Wiley, New York.
- 282 Grandy, A. S., and J. C. Neff. 2008. Molecular C dynamics downstream: The biochemical
 283 decomposition sequence and its impact on soil organic matter structure and function.
 284 *Science of The Total Environment* 404:297–307.
- 285 Haider, K., and J. P. Martin. 1981. Decomposition in soil of specifically ¹⁴C-labeled model and
 286 cornstalk lignins and coniferyl alcohol over two years as influenced by drying, rewetting,
 287 and additions of an available C substrate. *Soil Biology and Biochemistry* 13:447–450.
- 288 Hall, S. J., W. Huang, and K. E. Hammel. 2017. An optical method for carbon dioxide isotopes
 289 and mole fractions in small gas samples: Tracing microbial respiration from soil, litter,
 290 and lignin. *Rapid Communications in Mass Spectrometry* 31:1938–1946.
- 291 Hall, S. J., W. L. Silver, V. I. Timokhin, and K. E. Hammel. 2015. Lignin decomposition is
 292 sustained under fluctuating redox conditions in humid tropical forest soils. *Global*
 293 *Change Biology* 21:2818–2828.
- 294 Hammel, K. E., A. N. Kapich, K. A. Jensen Jr, and Z. C. Ryan. 2002. Reactive oxygen species as
 295 agents of wood decay by fungi. *Enzyme and Microbial Technology* 30:445–453.
- 296 Hernes, P. J., K. Kaiser, R. Y. Dyda, and C. Cerli. 2013. Molecular trickery in soil organic
 297 matter: hidden lignin. *Environmental Science & Technology* 47:9077–9085.

- 298 Huang, W., K. E. Hammel, J. Hao, A. Thompson, V. I. Timokhin, and S. J. Hall. 2019.
 299 Enrichment of lignin-derived carbon in mineral-associated soil organic matter.
 300 Environmental Science & Technology 53:7522–7531.
- 301 Kirk, T. K., and G. Brunow. 1988. Synthetic ^{14}C -labeled lignins. Methods in Enzymology
 302 161:65–73.
- 303 Kirk, T. K., and R. L. Farrell. 1987. Enzymatic "combustion": the microbial degradation of
 304 lignin. Annual Reviews in Microbiology 41:465–501.
- 305 Kleber, M. 2010. What is recalcitrant soil organic matter? Environmental Chemistry 7:320–332.
- 306 Klotzbücher, T., K. Kaiser, G. Guggenberger, C. Gatzek, and K. Kalbitz. 2011. A new
 307 conceptual model for the fate of lignin in decomposing plant litter. Ecology 92:1052–
 308 1062.
- 309 Klotzbücher, T., K. Kalbitz, C. Cerli, P. J. Hernes, and K. Kaiser. 2016. Gone or just out of
 310 sight? The apparent disappearance of aromatic litter components in soils. SOIL 2:325–
 311 335.
- 312 Kögel-Knabner, I., G. Guggenberger, M. Kleber, E. Kandeler, K. Kalbitz, S. Scheu, K.
 313 Eusterhues, and P. Leinweber. 2008. Organo-mineral associations in temperate soils:
 314 Integrating biology, mineralogy, and organic matter chemistry. Journal of Plant Nutrition
 315 and Soil Science 171:61–82.
- 316 Martin, J. P., K. Haider, and G. Kassim. 1980. Biodegradation and stabilization after 2 years of
 317 specific crop, lignin, and polysaccharide carbons in soils. Soil Science Society of
 318 America Journal 44:1250.
- 319 Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf
 320 litter decomposition dynamics. Ecology 63:621–626.

- 321 Moorhead, D. L., R. L. Sinsabaugh, B. H. Hill, and M. N. Weintraub. 2016. Vector analysis of
 322 ecoenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Biology*
 323 *and Biochemistry* 93:1–7.
- 324 Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling
 325 soil organic matter levels in Great Plains grasslands. *Soil Science Society of America*
 326 *Journal* 51:1173–1179.
- 327 Rousk, J., P. C. Brookes, and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial
 328 growth suggest functional redundancy in carbon mineralization. *Applied and*
 329 *Environmental Microbiology* 75:1589–1596.
- 330 Ruiz-Dueñas, F. J., and Á. T. Martínez. 2009. Microbial degradation of lignin: how a bulky
 331 recalcitrant polymer is efficiently recycled in nature and how we can take advantage of
 332 this. *Microbial Biotechnology* 2:164–177.
- 333 Talbot, J. M., and K. K. Treseder. 2012. Interactions among lignin, cellulose, and nitrogen drive
 334 litter chemistry–decay relationships. *Ecology* 93:345–354.
- 335 Tedersoo, L., M. Bahram, S. Põlme, U. Kõljalg, N. S. Yorou, et al. 2014. Global diversity and
 336 geography of soil fungi. *Science* 346:1078.
- 337 Thevenot, M., M.-F. Dignac, and C. Rumpel. 2010. Fate of lignins in soils: A review. *Soil*
 338 *Biology and Biochemistry* 42:1200–1211.
- 339 Waksman, S. A., and K. R. N. Iyer. 1932. Contribution to our knowledge of the chemical nature
 340 and origin of humus: I. On the synthesis of the “humus nucleus.” *Soil Science* 34:43–69.
- 341 Wood, S. 2017. *Generalized Additive Models: An Introduction with R*. 2nd edition. CRC Press,
 342 Boca Raton, FL.

Zak, D. R., W. A. Argiroff, Z. B. Freedman, R. A. Upchurch, E. M. Entwistle, and K. J. Romanowicz. 2019. Anthropogenic N deposition, fungal gene expression, and an increasing soil carbon sink in the Northern Hemisphere. *Ecology* 100:e02804.

366 **Figure captions:**

367 **Figure 1:** Conceptual models comparing bulk litter and lignin decomposition over time as mass
 368 remaining (a) or instantaneous fluxes (b). The *vertical grey line* indicates the transition from
 369 early- to late-stage decomposition. In the Berg and Staff (1980) model, lignin decomposition is
 370 low until unprotected litter C has been depleted but controls mass loss thereafter. In the
 371 Klotzbücher et al. (2011) model, lignin decomposition is greatest during early litter
 372 decomposition and subsequently declines relative to bulk litter decomposition.

373 **Figure 2:** Decomposition rates of lignin C_β (*orange circles*), litter C (*blue triangles*), and SOC
 374 (*green squares*) for each soil over time, expressed relative to initial C mass in each pool (note the
 375 logarithmic y-axis scale). Lines represent GAMM fits. The first two capital letters of each panel
 376 denote the state/province of soil origin, followed by the great group in the USDA soil taxonomy.

377 **Figure 3:** Pairwise relationships between instantaneous decomposition rates of litter C and lignin
 378 C_β for each soil. Numbers and shading denote the date of measurement. The *dashed line* indicates
 379 a 1:1 relationship.

380 **Figure 4:** Pairwise relationships between instantaneous decomposition rates of SOC and lignin
 381 C_β for each soil. Numbers and shading denote the date of measurement. The *dashed line* indicates
 382 a 1:1 relationship.