Reduction of forest soil respiration in response to nitrogen deposition

1   Revised Manuscript # NGS-12-01557

2

3   Reduction of forest soil respiration in response to nitrogen deposition

4

5   Authors :

6   I.A. Janssens, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610

7   Wilrijk, Belgium.

8   W. Dieleman, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610

9   Wilrijk, Belgium.

10   S. Luyssaert, LSCE CEA-CNRS-UVSQ, Orme des Merisiers, F-91191 Gif-sur-Yvette,

11   France.

12   J.-A. Subke, Stockholm Environment Institute, Department of Biology, University of York,

13   York, Y010 5DD, UK.

14   M. Reichstein, Max Planck Institute for Biogeochemistry, PO Box 100164, D-07701 Jena,

15   Germany.

16   R. Ceulemans, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610

17   Wilrijk, Belgium.

18   P. Ciais, LSCE CEA-CNRS-UVSQ, Orme des Merisiers, F-91191 Gif-sur-Yvette, France.

19   A.J. Dolman, Department of Hydrology and Geo-environmental Science, VU Amsterdam,

20   1081 HV Amsterdam, The Netherlands.

21   J. Grace, Institute of Atmospheric and Environmental Science, School of GeoSciences, The

22   University of Edinburgh, Edinburgh, EH9 3JN, Scotland, U.K.

23   G. Matteucci, CNR-ISAFOM, Via Cavour 4-6, I-87036 Rende, Italy.

24   D. Papale, Department of Forest Environment and Resources, University of Tuscia, I-01100

25   Viterbo, Italy.

26 S.L. Piao, Department of Ecology, Peking University, Beijing 100871, China.

27 E.-D. Schulze, Max Planck Institute for Biogeochemistry, PO Box 100164, D-07701 Jena,

28 Germany.

29 J. Tang, The Ecosystems Center, Marine Biological Laboratory, 7 MBL St, Woods Hole, MA

30 02543, USA.

31 B.E. Law, College of Forestry, Oregon State University, Corvallis, OR 97331-5752 USA.

32

33

34 **Main text**:

35   Total word count: 4000

36   Introductory paragraph: 135 words

37   Introduction: 321 words

38   Empirical evidence: 1663 words

39   Mechanisms: 1382 words

40   Implications: 499 words

41 100 references

42 Acknowledgments & Correspondence: 108 words

43 Figure legends

44 Box 1: includes 1 drawing !!!

45

46 **supplementary information:**

47 Materials & Methods, 2 Figures, 1 Table & 2 appendices

48

49 **In recent decades, many industrialized regions have been subject to enhanced**

50 **atmospheric deposition of reactive nitrogen. This unintended fertilization is recognized**

51 **to have stimulated forest growth, but nitrogen also strongly affects soil microbial**

52 **activity and thus the recycling of carbon and nutrients in soils. Here, we present**

53 **evidence from a meta-analysis that nitrogen deposition typically impedes carbon cycling**

54 **through both rhizospheric and saprophytic pathways, except where the additional**

55 **nitrogen remains insufficient to meet the nitrogen needs of trees and microbes. The**

56 **associated, nitrogen-induced reductions in respiratory carbon losses are often of the**

57 **same order of magnitude as the stimulation of wood production. These large reductions**

58 **have a substantial effect on the net exchange of carbon with the atmosphere. Whether**

59 **nitrogen deposition also affects soil carbon cycling in nitrogen-saturated and tropical**

60 **forests remains an open question.**

61

62

63

64 **1. Introduction**

65

66 Atmospheric deposition of reactive nitrogen (N), originating mainly from fossil fuel burning

67 and artificial fertilizer applications [1], has increased three- to five-fold over the last century [2].

68 In many areas of the globe, N deposition is expected to increase further [3,4], with global

69 deposition rates projected to increase by a factor of 2.5 by the end of the century [5].

70 Atmospheric deposition of N has many negative ecological effects in terrestrial and aquatic

71 ecosystems, such as eutrophication and loss of biodiversity [6-8]. Excessive N deposition can

72 also result in soil acidification, loss of base cations and nitrate leaching into groundwater [9-11].

73 However, low levels of atmospheric N deposition can have one positive effect: it stimulates

74   plant growth and the associated uptake of carbon (C) from the atmosphere contributes to

75   climate change mitigation [12-15]. In 2007, Magnani and co-workers even revealed N deposition

76   as the dominant driver of forest ecosystem carbon sequestration [16].

77

78   The publication by Magnani and colleagues generated an intense debate on the magnitude and

79   sustainability of the N-induced C sink and its underlying mechanisms [17-19]. Two responses

80   contribute to the enhanced C sink strength of forests under elevated atmospheric N

81   deposition: increased wood formation [13,14,20] and accumulation of surface litter and soil

82   organic matter (SOM) [14,21,22]. Accumulation of SOM could originate from increased soil C

83   inputs, but this is not commonly observed in forests exposed to N deposition [14]. In contrast,

84   decreased rates of plant litter and SOM decomposition by enhanced soil N inputs have

85   frequently been reported [23,24]. Through a meta-analysis of measurements in N-addition

86   experiments (Appendix S1) and a comparison of study sites exposed to elevated or

87   background atmospheric N deposition (Appendix S2), we show that the negative effect of N

88   on below-ground C fluxes is widespread, albeit not universal, in forest ecosystems. Despite

89   the long history of evidence that N deposition slows decomposition, this effect has not been

90   included in current C cycle models.

91

92

93

94

2. Empirical evidence for a reduction of soil C cycling by N

2.1. Litter quality modulates N effect on litter decomposition

It is well established that leaf litter with higher N (or high N to lignin ratios) decomposes faster than its lower N counterpart [25-27], although this difference may revert during later stages of the decomposition process [28]. However, adding N to low N litter does not accelerate its decomposition. In an early review of more than 60 experiments on the effect of N application on decomposition of various types of organic matter, Kåre Fog [23] concluded that "*when all these papers are considered together, it is evident that no effect, or even a negative effect, of the addition of N to decomposing organic material is a very widespread phenomenon*". Literature reviews have indicated distinct decomposition responses to N addition in litter with low versus high lignin content [23,29]. In agreement with earlier studies on leaf litter decomposition, N addition accelerates decomposition of low lignin litter. In contrast to the response of low-lignin litter, Fog [23] concluded that: "*in recalcitrant substrates the situation is completely different. Here negative effects of the addition of N are much commoner, especially if the lignin content is high, in direct contrast to the case above. Thus, the higher the lignin content, the smaller the amount of N tolerated*". Almost exactly the same results were obtained in a recent comprehensive review with quantitative meta-analysis on the effects of N addition on litter decomposition [29]. Moreover, the overall mean response to N addition tended to be positive in short-term studies, but a significant decrease in decomposition rate (17%) was detected in studies lasting longer than two years.

Given that forest litter contains a substantial fraction of low quality litter (litter with high lignin and low N contents that decomposes very slowly) and the clear evidence that low quality litter is negatively affected by N addition, it is to be expected that atmospheric N

120   deposition would typically reduce litter decomposition in forest ecosystems. Our statistical

121   meta-analysis [30] applied to data from 20 N-manipulation experiments in forests suggested

122   only a small and statistically insignificant decline in leaf litter decomposition rate (Fig. 1).

123   However, in agreement with the previous studies, we found that N addition tends to accelerate

124   decomposition in genera producing easily degradable leaf litter (e.g. *Acer, Populus, Betula*),

125   whereas it clearly reduced decomposition rates in species with more recalcitrant litter (*Pinus,*

126   *Picea, Fagus, Quercus*; Fig. S1). Moreover, all forests produce large quantities of lower

127   quality litter, such as twigs, branches, and seed pods, which may be even more strongly

128   affected by added N, but data are not available to test this hypothesis.

129

130

131   *2.2. Heterotrophic respiration is commonly reduced*

132

133   Respiration in aerobic soil involves the breakdown or organic molecules with, in aerobic soil,

134   CO_2 as the main end-product. Release of CO_2 is therefore commonly used as a proxy for

135   respiratory activity. Here, we define respiration by organisms obtaining their energy from the

136   decomposition of litter and SOM as heterotrophic respiration. Heterotrophic respiration is

137   thus an integrator of decomposition of organic matter in all stages of decomposition and in all

138   soil layers, making its response to N addition more relevant to soil C cycling than that of leaf

139   litter decomposition alone.

140   To mitigate variation due to methodological differences [31,32], we focus this review on

141   heterotrophic respiration estimates obtained either from lab incubation or with the trenching

142   technique [31], both of which physically separate the soil from the root inputs. Our statistical

143   meta-analysis revealed that the average response of heterotrophic respiration to N addition is

144   much more pronounced than that of leaf-litter decomposition alone. Averaged over 36 N-

145   manipulation studies in forest ecosystems, heterotrophic respiration declined by 15% when N

146   was added (Fig.1). Variation among experiments was very high, with responses ranging

147   between a reduction of 57% and stimulation by 63% (Fig. 2, left panel). Forests exposed to

148   elevated atmospheric N deposition are also observed to have lower heterotrophic respiration

149   than forests receiving background N deposition (wet deposition < 5.5 kg N ha^{-1} a^{-1}; Fig. 3).

150   For forests with an NPP around 600 g C m^{-2} a^{-1}, the reduction amounts to roughly 100 g C m^{-2}

151   a^{-1} (Fig. 3). The different slopes of the two regressions in Figure 3 further suggest that at

152   highly productive sites, where N is unlikely to be the most limiting nutrient, N deposition has

153   a stronger negative effect than at less productive sites, where N immobilization is likely

154   higher and the negative effect on heterotrophic respiration is only marginal.

155   It can be concluded that both episodic addition of high fertilizer amounts and chronic

156   deposition of small amounts of N induce a decline in heterotrophic respiration in most –but

157   not all– forest ecosystems.

158

159

160   *2.3. Soil CO2 efflux response depends on productivity response*

161

162   Soil CO_2 efflux (SCE) is an important indicator for belowground C cycling [33]. Although

163   heterotrophic respiration constitutes a substantial part of SCE, two important C fluxes, related

164   to the presence of roots in soils, differentiate SCE from heterotrophic respiration. First, a

165   major component of SCE is autotrophic in nature (root-, mycorrhizal- and rhizosphere

166   respiration), coupling temporal variation in SCE to that in belowground C allocation and,

167   ultimately, photosynthesis [34-36]. Second, rhizodeposition, the transfer of root-derived C

168   compounds to soil, can stimulate microbial activity and thus prime decomposition of SOM [37-

169   39]. This priming mechanism alters heterotrophic respiration, but could not have made a

170   contribution to the heterotrophic respiration measurements presented in section 2.2, which

171   were all made in the absence of live roots.

172

173   As with heterotrophic respiration, there is convincing evidence that SCE declines following N

174   addition, either through fertilization (-10%; Fig. 1) or through atmospheric N deposition (Fig.

175   4). In most studies, this negative effect appears almost instantaneously, while the negative

176   responses persist for years after the addition of nitrogen has ceased [24,40,41]. Although the

177   average response of SCE in fertilization experiments was clearly negative, SCE was found to

178   be positively affected by N addition in roughly 25% of the 57 manipulation studies (Fig. 2,

179   right hand panel). A closer look at the experiments where SCE increased following N

180   fertilization revealed that these were mainly studies where N addition has the potential to

181   strongly enhance photosynthesis: very young plantations (<4 years), where N addition may

182   have accelerated canopy development, and CO_2-fumigated forest stands, where extra N helps

183   to sustain the stimulatory CO_2 effect on photosynthesis (Figures 2 and S2; [42-44]). Part of the

184   increased C availability was probably allocated belowground, stimulating rhizosphere

185   respiration and microbial activity. We thus hypothesize that the increases in SCE observed in

186   a sub-set of studies (Fig. 2, right hand panel) are attributable to enhanced photosynthesis

187   following N enrichment.

188   Excluding the very young and CO_2-fumigated sites, the average decline in SCE amounted to –

189   17% (Fig S2), roughly 150-200 g C m^{-2} a^{-1}. Sites receiving more than 50 kg N ha^{-1} a^{-1}

190   exhibited a stronger decline in SCE (-21%) than sites receiving doses of less than 50 kg N ha^{-1}

191   a^{-1} which better mimics the effects of atmospheric N-deposition. Nevertheless, the response of

192   SCE in this latter group remained significantly negative (-10%). Per unit N added, this

193   reduction in SCE amounts to 36 g C per g N added. At a small number of sites older than 5

194   years and not enriched with CO_2, SCE responded positively (or did not respond) to N

195 addition. At these few sites, we speculate that N might be limiting microbial growth and thus

196 heterotrophic respiration (see section on heterotrophic respiration above).

197

198

199 *2.4. Soil C increases, microbial biomass decreases*

200

201 The response of plant-litter inputs (leaf-litter fall and fine-root production) to N addition is

202 variable; both increases and decreases are frequently observed, and on average litter inputs are

203 not affected (Fig.1). In N fertilization experiments, the combination of reduced heterotrophic

204 C losses and unaltered plant-litter inputs results in substantial soil C accumulation (relative

205 increase of 10%; Fig. 1), corresponding to 19 g C per g N added. Thus, on average, about half

206 of the reduction in SCE in the manipulation experiments may be due to C accumulating in the

207 soil. This substantial increase in soil C should be interpreted with caution, because most

208 studies report C pool changes only for the uppermost soil layers.

209 In a comprehensive review of results from ^{15}N field experiments, long-term low dose N

210 fertilizer experiments and ecosystem models, de Vries and co-workers [45] also reported that N

211 deposition substantially stimulated soil C sequestration. Taking all evidence into account, soil

212 C sequestration in European forests in response to N deposition was estimated to range from 5

213 to 23 g C g N^{-1} [45], agreeing well with our estimate. Sequestration rates of similar magnitudes

214 were also reported in other, more limited multi-site analyses [14,46].

215 The efficiency of soil C storage per unit N deposited declines with the amount of N added or

216 deposited to the forest [46]. This trend is expected, because as N availability increases, a larger

217 fraction of it will be lost to groundwater and atmosphere, and the probability for adverse

218 effects of N saturation increases proportionally.

219   Caution is, however, warranted when interpreting spatial correlations between N deposition

220   and soil C sequestration across forests (Peter Högberg, personal communication). Within

221   Europe, for example, N-deposition co-varies with natural soil nutrient availability. Human

222   population centres have developed in the more fertile regions with good climate for N

223   mineralization (not too dry, not too cold) and N deposition increased with population. Hence,

224   soil C accumulation may be partly attributable to the more favourable, natural nutrient

225   availability.

226

227   In agreement with the declining heterotrophic respiration and SCE, we observe a statistically

228   significant decline (-11%) in microbial biomass (Fig. 1). A review on the effects of N

229   deposition on microbial biomass showed that the average decline exceeded 20% in both

230   temperate and boreal forests [47]. When excluding very young forests and elevated CO_2

231   treatments from our analysis, we observe an average reduction of microbial biomass of 16%

232   (Fig. S1), in line with the review by Treseder [47] and with the response of SCE.

233

3. Processes underlying the reduction of soil respiration

Empirical evidence thus tells us that various aspects of belowground C cycling are negatively affected by N addition, that these effects are widespread, but that there are also conditions under which N addition accelerates decomposition and soil respiration (severe N-limitation, young forests, elevated CO_2 experiments). The following mechanisms have been put forward in the literature to explain these observations (Box 1).

3.1 Reduced belowground C allocation

Nitrogen is the most important macro nutrient. With the exception of regions where other nutrients are more limiting, a large increase in N availability to trees reduces the need for investment of C in their nutrient-absorbing system. This induces a shift in C allocation in favour of production of aboveground tissues at the expense of the root system [48]. In absolute values our data do not show clear differences in fine root NPP between N-fertilized and control plots (Fig. 1). However, the nutrient acquisition system is not limited to the root system and there is abundant evidence that N enrichment spectacularly affects the activity of the rhizosphere, and of mycorrhizal root symbionts in particular [49]. In a N-supply gradient study, C allocation to fungal symbionts was the process that responded most to N addition [50]. Drastic declines in the production of fruiting bodies by mycorrizal fungi[51], in the contribution of mycorrhizae to total microbial biomass [52], in mycorrhizal diversity (see Treseder [47] for review), in mycorrhizal infection rates and survival [53], and in arbuscular mycorrhizal biomass, hyphal length and storage structures [54,55] have all been reported in response to N addition. These results reflect reduced reliance of trees on fungal symbionts under high N deposition, which underlies the strong reduction in belowground C allocation.

259 The mere reduction in belowground C allocation has been shown to produce a direct negative

260 effect on rhizosphere respiration and thus SCE [56]. The reduction of C inputs into the soil is an

261 important mechanism via which also decomposition and soil respiration may be altered (Box

262 1).

263 Mycorrhizal root symbionts have the capacity to degrade organic matter [57] and their decline

264 would therefore be expected to directly reduce decomposition. Additionally, mechanisms

265 exist that are potentially even more relevant for the response of decomposition to the shift in

266 belowground C allocation. Fine roots and especially mycorrhizal hyphae exude substantial

267 amounts of soluble organic compounds [58] and these compounds serve as C and energy sources

268 for saprotrophic organisms that subsequently decompose organic matter in search of nutrients

269 [59]. This mechanism, known as the priming effect, is widely accepted to exert a major control

270 over soil organic matter decomposition [38,60-64]. By supplying less substrate to the

271 decomposers, the N-induced reduction of belowground C allocation can thus be expected to

272 impede decomposition of SOC.

273

274 The reduction in belowground C allocation thus contributes to the reduction in SCE (on

275 average 36 g C per g N; section 2.3), but cannot explain the substantial increase in soil C (on

276 average, 19 g C per g N; section 2.4). Such large soil C accumulation can only be explained

277 by other mechanisms directly reducing the activity of saprotrophs.

278

279

280 *3.2 Shifts in saprotrophic community structure or function*

281

282 There are multiple mechanisms through which alteration of microbial community structure or

283 function may affect decomposition of soil organic matter. First, addition of N could make

284   saprotrophic organisms switch from decomposing N-containing recalcitrant SOM to energy-

285   rich rhizodeposits (preferential substrate theory [63,65-67]). Alternatively, less efficient microbial

286   populations (less C assimilated in biomass and more CO_2 released per gram litter

287   decomposed) that require little N could be outcompeted by populations that are currently

288   nitrogen limited, but are more efficient [41]. Ågren and co-workers [41] postulated that such a

289   microbial community shift towards more efficient, N-demanding species could explain the

290   observed reductions in SCE. Their model would also be consistent with the observed

291   increases in soil C stocks and reduced microbial biomass if the new microbial populations

292   also produced more recalcitrant organic molecules.

293   In the early 1960's Mangenot & Reymond [68] had observed that by adding N to sawdust,

294   several species of wood-decomposing basidiomycetes lose their competitive ability, with

295   different species dropping out at sequentially higher N levels. There is ample recent evidence

296   that N addition to forest soils induces such shifts in microbial community composition [47,69-71].

297

298   Whatever the exact mechanism explaining the reduced decomposition of recalcitrant SOC or

299   of litter in final stages, alterations in the activity or expression of enzymes involved in the

300   decomposition process are likely to play a key role. Cellulose decomposing – and phosphate

301   acquiring enzymes were reported to increase following N fertilization, especially in N-limited

302   ecosystems [72-74], often accelerating cellulose decomposition [74,75]. In contrast, lignin-degrading

303   enzymes, such as phenol oxidases and peroxidases are frequently down-regulated [23,70,74,76,77],

304   but see [73]. Several white-rot fungi were found not to synthesize their lignin-degrading

305   enzymes in the presence of low molecular weight N compounds [24,78,79], but this response

306   varies among species [23,80]. One potential reason for this reduced synthesis of lignin-degrading

307   enzymes is that lignolitic fungi have low efficiency and growth rate, and are therefore likely

308   to be out-competed by other microbes, resulting in reduced ligninase activity and, hence,

309   lignin degradation [41,68,81]. Many easily decomposable substrates are embedded in the lignin

310   matrix within fresh plant litter [23]. Thus, by degrading lignin more slowly, decomposition of all

311   compounds within the lignin matrix is reduced [76].

312

313   These shifts in the expression and/or activity of various enzymes are thus likely to explain the

314   observation that more labile litter types or compounds (such as cellulose) decompose faster

315   following N addition, especially in the short term [23,29]. In the long-term, the reduced

316   production or activity of specific enzymes involved in the degradation of more recalcitrant

317   compounds may be responsible for the negative effect of N addition. Although the concepts

318   of altered microbial community structure and enzymatic spectra are appealing, Keeler et al [73]

319   were not able to find any correspondence between the N-addition effects on decomposition

320   rates and on the activities of six key-enzymes involved in decomposition. Moreover, despite

321   the strong evidence that lignin degrading enzymes are suppressed by N addition, this

322   mechanism may only be valid in organic surface layers; in mineral soils neither lignin nor

323   lignin-derivatives accumulate in soils exposed to N addition (*e.g.*[71]). It is thus obvious that the

324   molecular transformations in soils and the role of exo-enzymes in the degradation of the

325   produced recalcitrant compounds are far from elucidated.

326

327

328   *3.3 The role of stabilization mechanisms*

329   A major fraction of SOM is chemically or physically protected from microbial decay [82]. If N

330   addition were to interact with these stabilization mechanisms, a major effect on

331   decomposition and heterotrophic respiration could be expected. One hypothesis often brought

332   forward to explain the reductions in decomposition or heterotrophic respiration is abiotic

333   stabilization of SOM [23,24]. There is evidence of direct chemical incorporation of added

334 nitrogen into organic matter, producing heterocyclic forms of N (indoles and pyroles) [83] or

335 phenolic compounds polymerized by N-bridges [84], two groups of compounds that are highly

336 resistant to degradation by microbial enzymes. By chemically protecting part of the organic

337 matter available for decomposition, these abiotic reactions could thus reduce decomposition

338 and enhance C sequestration (see also [23,24]). However, ^{15}N NMR spectrometry indicates that

339 most of the retained N is in the amide form, indicating dominance of biotically mediated

340 formation of recalcitrant SOM [85-88], possibly in response to an N-induced shift in microbial

341 community composition[41]. Although this downplays the role of abiotic reactions postulated to

342 play an important role [23,24], it does not rule them out.

343 Incorporation of SOM within soil aggregates could also constitute an important physical

344 stabilization mechanism. Aggregate formation correlates positively with rhizodeposition and

345 microbial biomass and -activity, which all decline in response to N deposition. Hence,

346 aggregate formation is not expected to explain the reduced SOM cycling.

347 Finally, soil acidification is also a stabilization mechanism through which decomposition of

348 plant litter and SOM could be reduced. Given that soil pH is crucial to enzyme functioning [23],

349 acidification could have a detrimental effect on microbial activity and thus on decomposition

350 of SOM. Especially after chronic N deposition, poorly buffered soils where nitrification

351 occurs will tend to exhibit lower pH [23,89]. Nonetheless, even where soil acidification does not

352 occur, negative effects of N addition are frequently observed [23]. Hence, acidification may

353 aggravate the response, but not explain it completely.

354

355

356

4. Implications

Both the N-fertilization manipulation experiments and the comparison between forest ecosystems subjected to background versus elevated N deposition provide evidence for a decline in SCE and heterotrophic respiration of the same order of magnitude as net ecosystem productivity (the actual net CO_2 uptake or release by ecosystems) reported elsewhere[90-95]. It is thus not surprising that Magnani and co-workers [16] identified N deposition as a better determinant of forest net ecosystem productivity than climate or site productivity. Networks of ecosystem CO_2 flux measurements are commonly used to construct greenhouse gas balances [90] or to extract information on the determinants of these fluxes [16,91-95]. The fact that N deposition often reduces heterotrophic respiration and SCE by such vast amounts implies that syntheses of CO_2 flux networks have to take N deposition into account when analyzing effects of *e.g.* climate on C cycling.

Eventually, all forests receiving elevated N deposition for decades or longer, will run into N saturation, a state in which the forest N cycle is no longer closed [87]. Although the exact effects remain unclear, a decrease in productivity could be anticipated through the loss of base cations and lower phosphorus availability [9,11,96,97]. Long-term responses may thus differ from the overall response reported here.

To date, N deposition has been elevated mainly in regions with relatively young, nutrient-rich soils, where N is often limiting plant growth (Eastern US, Europe, China). It remains to be tested whether N deposition will affect C cycling (stimulate wood growth and retard SOM decomposition) similarly in tropical (but also other) regions with older, severely weathered soils, where N may not be the most limiting nutrient [98]. If N enrichment were to slow down

382   decomposition, then nutrient immobilization in accumulating SOC could negatively affect

383   tropical forest productivity. Because 70% of the global primary productivity is realized in the

384   tropics, a shift in N-deposition towards these regions might alter the global C-balance

385   differently to that which might be expected by extrapolating the responses in temperate

386   regions. Current understanding of C-nutrient interactions in severely weathered soils or soils

387   with shortages of other nutrients remains extremely limited and N-addition manipulation

388   studies in these areas too rare to allow statistical meta-analysis

389

390   The evidence for altered belowground C cycling presented here highlights the need to

391   incorporate N cycling and N deposition into terrestrial C cycle models; not only the potential

392   N effects on productivity and interactions with increasing atmospheric CO_2 levels [99,100], but

393   especially the negative effects on decomposition, belowground C allocation and the coupling

394   of C and N cycling in ecosystems. To do this properly, more work is needed to fully

395   understand the relative importance of the different mechanisms at play (Box 1), on the long-

396   term responses to chronic N deposition, as well as on the responses in tropical areas. Given

397   that the size of the N-induced inhibition of below-ground respiration is of the same order of

398   magnitude as the forest C sink, a better understanding of N deposition effects should be a

399   strong, future research priority.

400

401

402 **<u>References</u>**

403

404 [1]   E. A. Davidson, *Nature Geoscience* **2** (9), 659 (2009).

405 [2]   K.L. Denman, G. Brasseur, A. Chidthaisong et al., in *Climate Change 2007: The*

406   *Physical Science Basis. Contribution of Working Group I to the Fourth Assessment*

407   *Report of the Intergovernmental Panel on Climate Change*, edited by S. Solomon, D.

408   Qin, M. Manning et al. (Cambridge University Press, Cambridge, 2007).

409 [3]   J. N. Galloway, F. J. Dentener, D. G. Capone et al., *Biogeochemistry* **70** (2), 153

410   (2004).

411 [4]   F. Dentener, J. Drevet, J. F. Lamarque et al., *Global Biogeochemical Cycles* **20** (4)

412   (2006).

413 [5]   J. F. Lamarque, J. T. Kiehl, G. P. Brasseur et al., *Journal of Geophysical Research-*

414   *Atmospheres* **110** (D19) (2005).

415 [6]   J. D. Aber, K. J. Nadelhoffer, P. Steudler et al., *Bioscience* **39** (6), 378 (1989).

416 [7]   P. M. Vitousek, J. D. Aber, R. W. Howarth et al., *Ecological Applications* **7** (3), 737

417   (1997).

418 [8]   L. C. Maskell, S. M. Smart, J. M. Bullock et al., *Global Change Biology* **16** (2), 671

419   (2010).

420 [9]   W. de Vries, C. van der Salm, G. J. Reinds et al., *Environmental Pollution* **148** (2),

421   501 (2007).

422 [10]   N. B. Dise, J. J. Rothwell, V. Gauci et al., *Science of the Total Environment* **407** (5),

423   1798 (2009).

424 [11]   P. Högberg, H. B. Fan, M. Quist et al., *Global Change Biology* **12** (3), 489 (2006).

425 [12]   D. S. Reay, F. Dentener, P. Smith et al., *Nature Geoscience* **1** (7), 430 (2008).

426 [13]   P. Ciais, M. J. Schelhaas, S. Zaehle et al., *Nature Geoscience* **1** (7), 425 (2008).

427 14 K. S. Pregitzer, A. J. Burton, D. R. Zak et al., *Global Change Biology* **14** (1), 142

428 (2008).

429 15 R. Q. Thomas, C. D. Canham, K. C. Weathers et al., *Nature Geoscience* **3** (1), 13

430 (2010).

431 16 F. Magnani, M. Mencuccini, M. Borghetti et al., *Nature* **447** (7146), 848 (2007).

432 17 W. de Vries, S. Solberg, M. Dobbertin et al., *Nature* **451** (7180), E1 (2008).

433 18 M. A. Sutton, D. Simpson, P. E. Levy et al., *Global Change Biology* **14** (9), 2057

434 (2008).

435 19 I. A. Janssens and S. Luyssaert, *Nature Geoscience* **2** (5), 318 (2009).

436 20 J. A. Elvir, G. B. Wiersma, A. S. White et al., *Canadian Journal of Forest Research-*

437 *Revue Canadienne De Recherche Forestiere* **33** (5), 862 (2003).

438 21 P. Olsson, S. Linder, R. Giesler et al., *Global Change Biology* **11** (10), 1745 (2005).

439 22 R. Hyvönen, G. I. Ågren, S. Linder et al., *New Phytologist* **173**, 463 (2007).

440 23 K. Fog, *Biological Reviews of the Cambridge Philosophical Society* **63** (3), 433

441 (1988).

442 24 B. Berg and E. Matzner, *Environmental Reviews* **5**, 1 (1997).

443 25 J. M. Melillo, J. D. Aber, and J. F. Muratore, *Ecology* **63** (3), 621 (1982).

444 26 J. D. Aber and J. M. Melillo, *Canadian Journal of Botany-Revue Canadienne De*

445 *Botanique* **58** (4), 416 (1980).

446 27 M.J. Swift, O.W. Heal, and J.M. Anderson eds., *Decomposition in Terrestrial*

447 *Ecosystems.* (Blackwell Scientific Publications, Oxford, 1979).

448 28 C. McClaugherty and B. Berg, *Pedobiologia* **30** (2), 101 (1987).

449 29 M. Knorr, S. D. Frey, and P. S. Curtis, *Ecology* **86** (12), 3252 (2005).

450 30 M. S. Rosenberg, D. C. Adams, and J. Gurevitch, *Metawin: Statistical Software for*

451 *Meta-Analysis.* (Sinauer Associates, Inc. Sunderland, Massachusetts, 2000).

452 31 P. J. Hanson, N. T. Edwards, C. T. Garten et al., *Biogeochemistry* **48** (1), 115 (2000).

453 32 J. A. Subke, I. Inglima, and M. F. Cotrufo, *Global Change Biology* **12**, 921 (2006).

454 33 W. Kutsch, M. Bahn, and A. Heinemeyer eds., *Soil Carbon Dynamics. An Integrated*

455   *Methodology.* (Cambridge University Press, Cambridge, 2009).

456 34 A. Ekblad and P. Högberg, *Oecologia* **127** (3), 305 (2001).

457 35 D. A. Sampson, I. A. Janssens, J. C. Yuste et al., *Global Change Biology* **13** (9), 2008

458   (2007).

459 36 P. Högberg, A. Nordgren, N. Buchmann et al., *Nature* **411** (6839), 789 (2001).

460 37 S. Fontaine, S. Barot, P. Barre et al., *Nature* **450**, 277 (2007).

461 38 W. Cheng and D.W. Johnson, *Plant and Soil* **202**, 167 (1998).

462 39 Y. Kuzyakov, *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur*

463   *Pflanzenernahrung Und Bodenkunde* **165** (4), 382 (2002).

464 40 P. J. Martikainen, T. Aarnio, V. M. Taavitsainen et al., *Plant and Soil* **114** (1), 99

465   (1989).

466 41 G. I. Ågren, E. Bosatta, and A. H. Magill, *Oecologia* **128** (1), 94 (2001).

467 42 R. Oren, D.S. Ellsworth, K.H. Johnsen et al., *Nature* **411**, 469 (2001).

468 43 J. R. Butnor, K. H. Johnsen, R. Oren et al., *Global Change Biology* **9**, 849 (2003).

469 44 R. Ceulemans and M. Mousseau, *New Phytologist* **127**, 425 (1994).

470 45 W. de Vries, S. Solberg, M. Dobbertin et al., *Forest Ecology and Management* **258**

471   (8), 1814 (2009).

472 46 R. Hyvönen, T. Persson, S. Andersson et al., *Biogeochemistry* **89** (1), 121 (2008).

473 47 K. K. Treseder, *Ecology Letters* **11** (10), 1111 (2008).

474 48 C. M. Litton, J. W. Raich, and M. G. Ryan, *Global Change Biology* **13** (10), 2089

475   (2007).

476 49 K. K. Treseder, *New Phytologist* **164** (2), 347 (2004).

477   50   M. N. Högberg, E. Baath, A. Nordgren et al., *New Phytologist* **160** (1), 225 (2003).

478   51   A. Ruhling and G. Tyler, *Ambio* **20** (6), 261 (1991).

479   52   A. Tietema, *Forest Ecology and Management* **101** (1-3), 29 (1998).

480   53   L. M. Egerton-Warburton and E. B. Allen, *Ecological Applications* **10** (2), 484 (2000).

481   54   N. C. Johnson, *Ecological Applications* **3** (4), 749 (1993).

482   55   L. T. A. van Diepen, E. A. Lilleskov, K. S. Pregitzer et al., *New Phytologist* **176** (1),
483   175 (2007).

484   56   R. P. Phillips and T. J. Fahey, *New Phytologist* **176**, 655 (2007).

485   57   R. L. Gadgil and P. D. Gadgil, *Nature* **233** (5315), 133 (1971).

486   58   M. N. Högberg and P. Högberg, *New Phytologist* **154** (3), 791 (2002).

487   59   K. J. van Groenigen, J. Six, B. A. Hungate et al., *Proceedings of the National*
488   *Academy of Sciences of the United States of America* **103**, 6571 (2006).

489   60   M. R. Hoosbeek, M. Lukac, D. van Dam et al., *Global Biogeochemical Cycles* **18**
490   (2004).

491   61   J. Wu, P. C. Brookes, and D. S. Jenkinson, *Soil Biology & Biochemistry* **25** (10), 1435
492   (1993).

493   62   Y. Kuzyakov, J. K. Friedel, and K. Stahr, *Soil Biology & Biochemistry* **32**, 1485
494   (2000).

495   63   S. Fontaine, G. Bardoux, L. Abbadie et al., *Ecology Letters* **7** (4), 314 (2004).

496   64   J. A. Subke, V. Hahn, G. Battipaglia et al., *Oecologia* **139** (4), 551 (2004).

497   65   R. Merckx, A. Dijkstra, A. Denhartog et al., *Biology and Fertility of Soils* **5** (2), 126
498   (1987).

499   66   L. Lekkerkerk, H. Lundkvist, G. I. Agren et al., *Soil Biology & Biochemistry* **22** (2),
500   161 (1990).

501   67   E. Liljeroth, J. A. Vanveen, and H. J. Miller, *Soil Biology & Biochemistry* **22** (8), 1015

502   (1990).

503   68   F. Mangenot and G. Reymond, *Revue Générale de Botanique* **70**, 107 (1963).

504   69   J. E. Compton, L. S. Watrud, L. A. Porteous et al., *Forest Ecology and Management*

505   **196** (1), 143 (2004).

506   70   S. D. Frey, M. Knorr, J. L. Parrent et al., *Forest Ecology and Management* **196** (1),

507   159 (2004).

508   71   X. J. Feng, A. J. Simpson, K. P. Wilson et al., *Nature Geoscience* **1** (12), 836 (2008).

509   72   R. L. Sinsabaugh, M. E. Gallo, C. Lauber et al., *Biogeochemistry* **75** (2), 201 (2005).

510   73   B. L. Keeler, S. E. Hobbie, and L. E. Kellogg, *Ecosystems* **12** (1), 1 (2009).

511   74   M. M. Carreiro, R. L. Sinsabaugh, D. A. Repert et al., *Ecology* **81** (9), 2359 (2000).

512   75   G. Sjoberg, S. I. Nilsson, T. Persson et al., *Soil Biology & Biochemistry* **36** (11), 1761

513   (2004).

514   76   J. L. DeForest, D. R. Zak, K. S. Pregitzer et al., *Soil Biology & Biochemistry* **36** (6),

515   965 (2004).

516   77   K. R. Saiya-Cork, R. L. Sinsabaugh, and D. R. Zak, *Soil Biology & Biochemistry* **34**

517   (9), 1309 (2002).

518   78   P. Keyser, T. K. Kirk, and J. G. Zeikus, *Journal of Bacteriology* **135** (3), 790 (1978).

519   79   M. Tien and S. B. Myer, *Applied and Environmental Microbiology* **56** (8), 2540

520   (1990).

521   80   M. P. Waldrop and D. R. Zak, *Ecosystems* **9** (6), 921 (2006).

522   81   J. L. DeForest, D. R. Zak, K. S. Pregitzer et al., *Soil Science Society of America*

523   *Journal* **68** (1), 132 (2004).

524   82   J. M. Oades, *Plant and Soil* **76** (1-3), 319 (1984).

525   83   K. A. Thorn and M. A. Mikita, *Science of the Total Environment* **113** (1-2), 67 (1992).

526   [84]   H. Nömmik and K. Vahtras, *Agronomy Monographs* **22**, 123 (1982).

527   [85]   J. Burdon, *Soil Science* **166** (11), 752 (2001).

528   [86]   P. W. Clinton, R. H. Newman, and R. B. Allen, *European Journal of Soil Science* **46**

529   (4), 551 (1995).

530   [87]   J. Aber, W. McDowell, K. Nadelhoffer et al., *Bioscience* **48**, 921 (1998).

531   [88]   R. Sutton and G. Sposito, *Environmental Science & Technology* **39** (23), 9009 (2005).

532   [89]   R. D. Bowden, E. Davidson, K. Savage et al., *Forest Ecology and Management* **196**

533   (1), 43 (2004).

534   [90]   E. D. Schulze, S. Luyssaert, P. Ciais et al., *Nature Geoscience* **2** (12), 842 (2009).

535   [91]   I. A. Janssens, H. Lankreijer, G. Matteucci et al., *Global Change Biology* **7** (3), 269

536   (2001).

537   [92]   P. Ciais, M. Reichstein, N. Viovy et al., *Nature* **437** (7058), 529 (2005).

538   [93]   S. Luyssaert, I. Inglima, M. Jung et al., *Global Change Biology* **13**, 2509 (2007).

539   [94]   M. Reichstein, P. Ciais, D. Papale et al., *Global Change Biology* **13** (3), 634 (2007).

540   [95]   S. L. Piao, P. Ciais, P. Friedlingstein et al., *Nature* **451** (7174), 49 (2008).

541   [96]   E. D. Schulze, R. Oren, and O.L. Lange, in *Processes leading to forest decline.*, edited

542   by E. D. Schulze, O.L. Lange, and R. Oren (Springer, Berlin Heidelberg, 1989), Vol.

543   77, pp. 460.

544   [97]   S. Braun, V.F.D. Thomas, R. Quiring et al., *Environmental Pollution* (in press).

545   [98]   P. A. Matson, W. H. McDowell, A. R. Townsend et al., *Biogeochemistry* **46** (1-3), 67

546   (1999).

547   [99]   G. Bonan, *Nature Geoscience* **1** (10), 645 (2008).

548   [100] S. Zaehle, P. Friedlingstein, and A. D. Friend, *Geophysical Research Letters* **37**.

549   All correspondence and requests for materials can be send to: ivan.janssens@ua.ac.be

550

551

560

561

562   IAJ, WD and SL conceived the manuscript. SL, WD, JAS, II and BEL provided the data. WD

563   and SL performed the analyses. All authors collaborated in the writing.

564

Figure 1: Effect of experimental N-addition on various forest C pools and fluxes as calculated by meta-analysis. Positive values indicate that N addition increased the factor, negative values indicate a decrease. Error bars indicate the 95% confidence interval. Data are the weighted means for n data points (n is listed along the righthand axis). Parameters listed are C inputs: litterfall (LF) and fine-root production (FRP), C pools: total tree biomass (TB), microbial biomass (Cmic) and soil C content (soil C), and C losses: litter decomposition (LD), heterotrophic respiration (Rh), root respiration (Rr) and soil CO_2 efflux (SCE). Exact numbers can be found in Table S1.

Figure 2: Relative effect of N addition on heterotrophic respiration (Rh, left panel) and soil CO_2 efflux (SCE, right panel) in the manipulation experiments included in the statistical meta-analysis (circles) and their overall mean effect size (open red squares). Open black circles are forest stands more than four years old that received no additional CO_2 and their mean is represented by the open black square. Grey symbols are forest stands more than four years old exposed to elevated atmospheric CO_2 concentrations, solid black symbols are forests of four years old or younger, and solid red symbols are forest stands of four years old or younger that also received CO_2 fumigation. Error bars indicate the 95% confidence interval. In these young and/or CO_2 fumigated forests, N deposition favours SCE (positive response), whereas in forests older than 5 years, N deposition has a negative effect on SCE almost consistently. The effect of N addition on Rh, in contrast, is more consistently negative and does not differ between very young and/or CO_2 fumigated and older forests. Site information, data and references to all studies included in this analysis are given in Appendix S1.

590   Figure 3: Observed annual heterotrophic respiration rates (Rh) as a function of annual

591   biomass production (NPP) in forests exposed to elevated or background N deposition. Open

592   black symbols and dotted black line (Y = 212 + 0.32 X) represent forests with wet N

593   deposition < 5.5 kg N ha^{-1} a^{-1} (the current average deposition rate in unaffected forests). Solid

594   red symbols and solid red line (Y = 217 + 0.16 X) reflect forests with N deposition > 5.5 kg N

595   ha^{-1} a^{-1} (affected by N deposition). The shaded areas surrounding the regression lines

596   represent the 67% confidence intervals (1 SD). The data support the hypothesis that intercepts

597   are equal (p = 0.475) but reject the hypothesis that slopes are equal (p = 0.965).

598

599   Figure 4: Observed annual soil CO_2 efflux (SCE) as a function of annual biomass production

600   (NPP) in forests exposed to elevated or background N deposition. Open black symbols and

601   dotted black line (Y = 153 + 1.47 X) represent forests with wet N deposition < 5.5 kg N ha^{-1}

602   a^{-1} (the current average deposition rate in unaffected forests). Solid red symbols and solid red

603   line (Y = 483 + 0.32 X) reflect forests with N deposition > 5.5 kg N ha^{-1} a^{-1} (affected by N

604   deposition). The shaded areas surrounding the regression lines represent the 67% confidence

605   intervals (1 SD).

606

607

608 **Box 1**

609

610 *Insert figure around here …*

611

612 Box 1: conceptual scheme depicting the mechanisms that explain the N-induced response of

613 below-ground C cycling and its variation (see also main text). The effects of N deposition on

614 the saprotrophic system may be related to two, mutually non-exclusive mechanisms: (1)

615 enhanced chemical stabilization of organic matter into compounds recalcitrant to microbial

616 decay (magenta arrows) and (2) shifts in microbial enzyme synthesis and activity towards

617 preferential decomposition of labile, energy-rich compounds, coupled with reduced

618 decomposition of recalcitrant substrates (brown arrows). In the presence of roots, N-effects

619 via altered rhizosphere C inputs (green arrows) influence rhizosphere respiration, but also C

620 cycling through the saprotrophic system. Under increasing N availability, wood production is

621 typically promoted at the expense of belowground C allocation, reflecting the reduced need

622 for an elaborate nutrient acquisition system. Declining availability of energy-rich compounds,

623 combined with excess nitrogen affects the functioning of the microbial community, producing

624 a different enzyme spectrum. The reduced C available for mineralization results in further

625 reduced saprotrophic biomass, and the associated increase in net N mineralization thus

626 aggravates the above-mentioned, direct negative N effects on decomposition of recalcitrant

627 soil organic matter.

628 Although retarded belowground C cycling in response to N addition is the general rule in

629 forests, there are exceptions where soil respiration is enhanced by N enrichment. In severely

630 N-limited forests, such as in the northern boreal zone, microbial biomass may grow following

631 N addition, resulting in enhanced enzyme production and increased soil respiration. More

632 commonly, however, N deposition may stimulate canopy photosynthesis. In N-limited, open

633   forests, as well as in very young, accruing plantations, N deposition can accelerate canopy

634   closure and thus enhance light interception and photosynthesis. In N-limited systems (*e.g.*

635   boreal forests or CO_2-enriched trees), leaf-level photosynthesis may also be enhanced by N

636   addition. This increase in photosynthesis may offset the relative decline in belowground C

637   allocation, such that more labile C enters the soil, fueling not only rhizosphere microbes but

638   also saprotrophic microbes and their decomposition of recalcitrant SOC. Hence, although

639   negative responses to N deposition and addition clearly dominate the literature, positive

640   responses do occur.

641   The scheme presented in this Box 1 is, however, not valid for N-saturated systems where

642   adverse effects such as acidification, cation leaching and altered vegetation composition may

643   induce very different responses.

644

645

646