



**SDI Review Form 1.6**

**PART 1:**

Journal Name:	<b><u>International Journal of Plant &amp; Soil Science</u></b>
Manuscript Number:	<b>2013_ IJPSS 4233</b>
Title of the Manuscript:	<b>Role of soil nitrogen for the conifers of the boreal forest: a critical review</b>
Type of the Article	<b>Review Paper</b>

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**(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)**

- This form has total 7 parts. Kindly note that you should use all the parts of this review form.



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### PART 2: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p>The authors have taken on a enormous task in reviewing the nitrogen literature pertaining to forests, a topic with many hundreds of citations at least from the last couple of decades. Having worked in this area for over a decade I can appreciate the challenge in pulling all of these topics into one critical review. Each of these topics – overview of the N cycle, N uptake and nutrition, N metabolism, N deposition – could each be the subject of a critical review.</p> <p>I think as a consequence of including so many topics, the authors were forced to rely on some broad and perhaps traditional generalizations about boreal forests that greatly undermine the interesting details in the ecology of these systems.</p>	<p>We are very grateful to both of the reviewers for spending so much of their time on our manuscript. We are to hear that both the reviewers agree that the article is interesting and appropriate for publication in “International Journal of Plant and Soil Science”, after some revisions. We have gone through each of the comments from the reviewers and have presented below our responses. The modifications are highlighted in yellow in the new version of the manuscript. Some paragraphs and sentences of the old manuscripts were completely suppressed in order to address specific advices to reduce the complexity of the review. The lines and paragraphs suppressed are indicated referring to the original version of the manuscript.</p> <p>Lupi et al. responses to Reviewer JK_MA</p> <p>The manuscript has been revised in order to reduce its complexity and focus more on a subset of topics, as suggested by one of the reviewers. See the revised manuscript</p>



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	<p>There were a few points, or hypotheses really, in section 6 about succession and N cycles that I would argue are not well supported.</p> <p>My advice would be to reduce the complexity of the review and focus in on a subset of topics to better cover your ideas; it is probably unwise to attempt to cover all N topics for all people.</p> <p>Some specific comments -</p> <p>Lines 11-16. Fire can remove hundreds of kg N per ha, whereas DON leaching is in the order of a few kg per ha. Surely wildfire deserves far more attention as an output of N.</p>	<p>(text highlighted in yellow) and the Optional/general Comments part (below) for more detailed information.</p> <p>Section 6 was rewritten and comments about succession and N cycles were stated differently (see Section 6, for example, lines 672-687).</p> <p>Some parts on N assimilation, which were considered less interesting and pertinent for the review, were deleted. See the revised manuscript (text highlighted in yellow) and the Optional/general Comments part (below) for more detailed information.</p> <p>The text has been modified accordingly. Lines 13-14 of the new manuscript: "...but in unperturbed forests DON relatively gains importance over mineral N, with losses in the order of a few kg per ha per year."</p> <p>And lines 16-19 "Fires (wildfire and broadcast burning) may indeed represent an important punctual output of N (hundreds of kg N per ha) and the fire return interval is important in determining</p>
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		<p>the long-term impact of N volatilization by fire on the long-term N balance of the ecosystem.”)</p> <p>See also additional lines in Table 1, about wildfire as an output of N and section 4.2 (Forest harvesting, fire and climate change: the impact of different anthropic and natural disturbances on the N cycle) Lines 464-495.</p>
	<p>Also, in Table 1, if this is to a comprehensive review then there should be more data on estimates for biological N fixation and volatilization to match the good detail on N deposition and leaching. Estimates of biological N fixation could include alder shrubs (e.g. <i>Alnus viridis</i>), legumes (<i>Lupinus</i> spp.) and <i>Suillus</i> tuberculatus; volatilization should include wildfire and managed fire (broadcast burning).</p>	<p>Additional information and estimates on Biological Nitrogen Fixation and volatilization through fire was added to Table 1</p>
	<p>Lines 59-62. Boreal landscapes with complex topography can have a range of N forms, including</p>	<p>These sentences were rewritten to avoid oversimplification and include the</p>



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	<p>NO<sub>3</sub><sup>-</sup> on the most productive forest ecosystems (fine-textured fluvial terraces, toeslopes). There is probably less nitrate in boreal systems as a whole compared to temperate forests, but your comments here are an oversimplification. See</p> <p>Giesler, R., Högberg, M., Högberg, P., 1998. Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. <i>Ecology</i> 79: 119-137; Högberg, M.N., Myrold, D.D., Giesler, R., Högberg, P., 2006. Contrasting patterns of soil N-cycling in model ecosystems of Fennoscandian boreal forests. <i>Oecologia</i> 147: 96-107.;</p> <p>Nordin, A., Högberg, P., Näsholm, T., 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. <i>Oecologia</i> 129: 125-132;</p> <p>Kranabetter, J.M., Dawson, C. and Dunn, D. 2007. Indices of dissolved organic</p>	<p>Reviewer's comments on topography and the range of N forms. Suggested references where integrated in the text.</p> <p>See lines 63-70 of the revised manuscript:</p> <p>“Boreal landscapes with complex topography can show a range of N forms ([63], [64], [65], [66]). When soils extraction are carried out, NH<sub>4</sub><sup>+</sup> is generally the predominant inorganic form of N in mature conifer stands on less fertile sites, while NO<sub>3</sub><sup>-</sup> tends to gain importance on the most productive forest ecosystems (e.g. toeslopes, fine-textured fluvial terraces), after disturbances (e.g. producing forest gaps, with temperature, pH and light conditions stimulating mineralization and nitrification) or in areas subject to high N depositions ([65], [67], [68]).</p>
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	<p>nitrogen, ammonium and nitrate across productivity gradients of boreal forests. Soil Biol. Biochem. 39: 3147-3158.</p> <p>Line 54. The term ‘late-successional mature boreal forests’ can refer to two distinct types of ecosystem change. The species replacement illustrated in Figure 2, where deciduous shrubs give way eventually to spruce stands, has undoubtedly strong influence on N cycles because of the complete change in litter quality and microclimate. However, in the boreal forest there is also abundant areas under secondary succession, where tolerant species such as spruce and fir establish immediately after a disturbance and persist through to the next stand-replacing event. In this scenario there might be pioneer shrubs and tree species in the mix but they drop out over time rather than initially dominate and be replaced.</p>	<p>The sentence has been changed and the term “late-successional mature boreal forests” substituted with a different phrase. See lines 57-60 of the revised manuscript: “Moreover, with increasing time since fire, in old forest stands, lower soil pH and the accumulation of organic matter with high concentrations of polyphenols may limit N mineralization, especially nitrification, with proteolysis (i.e. depolymerization of proteins) potentially gaining more importance (Fig. 2; [2], [52], [61])”</p> <p>The commentaries of the reviewers concerning the likely differences between primary and secondary succession have</p>
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	<p>This latter form of succession is probably most common, and I would think the authors might consider both pathways in describing N dynamics of late-successional stands as they probably differ substantially.</p> <p>In addition to trees, there is the fundamental role of carpet mosses in boreal forests, and the authors might want to describe how the establishment of mosses during forest succession could influence N</p>	<p>been integrated into the revised manuscript. For example, lines 74-80 “However important differences may exist between primary and secondary succession, with dramatic species changes in the former (and complete changes in litter quality and microclimate) and the same species composition sometimes maintained in the latter (e.g. black spruce and balsam fir establishing immediately after a disturbance and persisting to the next stand-replacing event), probably affecting differently the N cycle ([71], [70]). During secondary succession, pioneer shrubs and trees might drop out over time, rather than dominate and being replaced. Certainly more papers are needed to clarify the differences in N cycle between primary and secondary succession.”</p> <p>The suggested article has been integrated in the manuscript. A discussion on the role of N-fixation by mosses and their relation with N depositions and N availability has been included in section 2.1.1 (N-depositions and canopy N uptake: another</p>
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	<p>dynamics – e.g., Nilsson and Wardle 2005. Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. <i>Frontiers in Ecology and the Environment</i> 3: 421–428.</p> <p>Line 61. A good reference on forest disturbance and N for the boreal would be Jerabkova L, Prescott CE, Titus BD, Hope GD, Walters MB, 2011. A meta-analysis of the effects of clearcut and variable-retention harvesting on soil nitrogen fluxes in boreal and temperate forests. <i>Canadian Journal of Forest Research</i> 4: 1852-1870. Some of these numbers could be added to Table 2.</p> <p>Line 63. A recent paper on this point</p>	<p>important pathway?). Lines 240-252 of the revised manuscript.</p> <p>This reference and others have been integrated in the revised manuscript. Section 4.2 (Forest harvesting, fire and climate change: the impact of different anthropic and natural disturbances on the N cycles) has been added under Section 4 (Anthropic and natural factors affecting the N-cycle): see Lines 464-495 (for the part on fire and harvesting impacts) Moreover, section 5.4 (Disturbances, N-depositions and stand development: implications for N cycling) is maintained, with minor modifications (see lines 628-659).</p> <p>This article and others have been integrated</p>
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	<p>LeDuc SD, Rothstein DE. 2010. Plant-available organic and mineral nitrogen shift in dominance with forest stand age. Ecology 91:708-720. Probably the only paper I know of that makes this point, certainly more studies are needed to substantiate this is a universal pattern.</p> <p>Line 76. The ‘great losses’ of <math>\text{NO}_3^-</math> is followed by the comment that ‘high <math>\text{NO}_3^-</math> losses are rare or limited’ – seems contradictory.</p>	<p>in the revised manuscript. See lines 487-492 of the revised manuscript.</p> <p>The sentence has been modified to underline the situations in which nitrate losses may be more important and to state that these situations are limited to particular conditions in the boreal forest. See lines 94-101 of the revised manuscript “The <b>different</b> mobility of N <b>forms</b>, in particular <math>\text{NO}_3^-</math>, can lead to <b>greater losses than <math>\text{NH}_4^+</math></b>, for example during snowmelt, when low soil temperature, high water fluxes, and the long period of tree</p>
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	<p>Again, in line 81, the dramatic losses in site N through fire is left almost as an afterthought, which makes no sense to me when you consider</p>	<p>dormancy limit N uptake ([3], [30]). However, in the boreal forest, high <math>\text{NO}_3^-</math> losses are <b>probably</b> rare, <b>given the strong N sinks generally represented by soil and plants in this N-limited environment.</b> <b>Important N losses (e.g. N volatilization by fire and nitrate leaching following fire or clearcut) may be related to disturbances, especially when plant uptake is absent or reduced,</b> while DON losses (which are generally greater than DIN losses <b>in older undisturbed stands</b>) proportionally gain importance during the later phases of stand succession, when organic matter accumulates ([10], [76], [77]).”</p> <p>The role of fires has been précised, according to the Reviewer’s comments. See lines 101-103: “N losses in gaseous forms <b>during fires can</b></p>
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	<p>the actual values of N in flux between these processes.</p> <p>Lines 549-561. This sweeping generalization needs a more critical discussion as mentioned previously. I just find this dismissal of <math>\text{NO}_3^-</math> hard to accept, surely rich fluvial boreal ecosystems with productive spruce stands are exploiting nitrate as a N source.</p>	<p>be in the order of hundreds of <math>\text{kg N ha}^{-1}</math>, but episodic and with different impacts on long-term N trends depending on fire return interval and fire severity ([53]).”</p> <p>The sentences were rewritten in order to avoid oversimplification and allow more critical discussion. Comments on the variety of N forms in relation to various factors (including topography) have been added (see response to a similar commentary above). For this specific comment, see lines 523-528 of the revised manuscript: “There is evidence that in boreal soils, <math>\text{NO}_3^-</math> levels are often very low (except in the more fertile soils, in particular topographical situations and in the first years following disturbance) while <math>\text{NH}_4^+</math> and organic N usually represent the majority of the N pool, with an increase in the ratio of organic to inorganic N, late in the secondary succession, with the accumulation of organic matter, or along a</p>
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	<p>Line 563-589. Another potential issue with controlled studies is that the role of ectomycorrhizal is largely generic, with no ability to control the assemblage of fungal species on a root system. There is a considerable diversity in functional attributes among ectomycorrhizal species (Chalot, M., and Passard, C. 2011. Ectomycorrhiza and nitrogen provision to the host tree. In Ecological Aspects of Nitrogen Metabolism in Plants. 1<sup>st</sup> Edition. Eds J.C. Polacco and C.D. Todd. John Wiley &amp; Sons), and it may be argued that the species composition of ectomycorrhiza facilitate uptake of whatever predominant form of N is available in soils.</p> <p>Line 641. A recent publication on this point could be helpful Coates, K.D., Lilles, E.B., and Astrup, R. 2013. Competitive interactions across a soil fertility gradient in a multispecies forest. Journal of</p>	<p>decreasing gradient of site productivity ([2], [66], [97]). NO<sub>3</sub><sup>-</sup> was thus not considered the most important source of N for conifers of the majority of the boreal forest.”</p> <p>These comments have been integrated into the revised text. See lines 554-557 of the revised manuscript.</p> <p>This point was added to the revised manuscript. Lines 624-627: “In a recent publication, Coates et al. ([189]) demonstrated that the effect and</p>
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	<p>Ecology.</p> <p>Line 685, 691. In my opinion there is not much evidence to substantiate these comments. The vast number of silvicultural studies have reported a short-term assart flush of N followed by little to no mid- to long-term effects of clearcutting (Jerabkova et al. 2011), making it hard to contend that late-seral stands are fundamentally poorer in N availability. See also Smithwick et al. 2009 Long-term nitrogen storage and soil nitrogen availability in post-fire lodgepole pine ecosystems. Ecosystems 12:792-806 for a thoughtful discussion on long-trends in N availability.</p>	<p>importance of soil fertility in sub-boreal forests on radial growth of different tree species was context-dependent, affected by competition in species-specific manner and varying with the composition of local neighbourhood of the tree.”</p> <p>The comment at lines 685-86 of the previous manuscript has been removed.</p> <p>The sentence at line 691 of the previous manuscript has been rewritten in order to respond to the comments by the reviewer.</p> <p>Our point is not that mature stands have less N in the soil than younger stands (maybe the contrary is true), but that this N is less available since there is an increased proportion of N complexed with phenolic compounds, soil pH tends to decrease with time since fire and the organic matter accumulates with an decreasing proportion of N being represented by more readily available inorganic N.</p> <p>The first paragraph of section 6 (lines 672-678 of the revised manuscript) has</p>
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	<p>Furthermore, the supposed difference in decomposition rates of old-growth stands has not held up to scrutiny – Prescott C 2005. Do rates of litter decomposition tells us anything we really need to know? Forest Ecology and Management 220: 66-74.</p>	<p>been modified as follows:</p> <p>“The majority of the studies support the hypothesis that the growth of the boreal forest is limited by the availability of N. Nonetheless, the availability of N may change and be more limited in the late stages of stand development, when low rates of decomposition and mineralization cause an accumulation of organic matter on the soil. Organic N is less available to conifers than inorganic N, which predominates after disturbances (even in forest gaps, if above a certain size, [68]). Consequently, conifers of older forests probably rely more on organic N than earlier in the stand development, closer to the disturbance at the origin of the new stand.”</p> <p>We certainly agree with the point presented in the article cited by the reviewers that correlation and regression analyses do not indicate cause and effect. However, many studies have reported slow decomposition of litter and soil organic matter in boreal conifer forests (some are cited in the article suggested by the reviewer: Nilsson and Wardle 2005 Understory vegetation as a forest ecosystem driver: evidence from the</p>
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	<p>The effect of ‘time’ in all these studies is so minute that it is unlikely the literature would support early or late-seral conifers utilizing</p>	<p>northern Swedish boreal forest. <i>Frontiers in Ecology and the Environment</i> 3: 421–428.). However, we added a study by Prescott et al. (2003) at lines 474-477, which agrees with the conclusions of Prescott (2005), in which the rate of decomposition of litter and forest floor was similar in forest gaps of all sizes. Indeed, the question is not if litter decomposition is important, but if its rate is critical (as stated in Prescott (2005) “The <i>process</i> of litter decomposition is undeniably critical for maintaining site fertility and productivity, but is there evidence that the <i>rate</i> of litter decomposition is critical?”). We tried to avoid a direct reference to decomposition <i>rates</i> in the revised manuscript. However we present some studies observing negative responses of decomposition to N additions explained through observed repression of the lignolytic enzymes of decomposing fungi (lines 445-447; Prescott et al. 2000 <i>Forest Ecology and Management</i> 133(1-2):23-36 and Knorr et al. 2005 <i>Ecology</i> 86(12):3252-3257).</p> <p>However some articles observed an increase in the proportion of organic N over mineral N with increasing time since</p>
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	<p>predominantly different forms of N.</p> <p>There is also the point made earlier concerning secondary succession, and the authors should recognize that the majority of boreal forests undergo adjustments in species composition over time, not complete species replacement.</p> <p>Line 706. Although the author's hypothesis on N as a successional driver is an interesting idea, any of these arguments has to recognize and accomodate the well established role of light in forest succession (see for example Messier et al. 1999. Functional ecology of advance regeneration in relation to light in boreal forests. Can J For Res 29 812-823). It seems well established that shade-</p>	<p>disturbance and the accumulation of organic matter (e.g. Nilsson and Wardle 2005; Kielland et al. 2007). So we present these findings and we advance an hypothesis that conifers of young and mature stands may have access to different N forms or, better, to the same N forms but in different proportions.</p> <p>This point has been addressed; see response to a similar comment above. For example, lines 74-80 of the revised manuscript.</p> <p>Indeed N is not the sole factor involved in changes associated with forest succession. Of course the role of light is fundamental in determining the survival and growth response of advance regeneration in boreal forests, together with factors linked to the type and availability of adequate substrates and to biotic factors accounting for example for intra- and inter-specific competition. Line 706 of the previous version of the manuscript has been substituted by line 690 in the revised manuscript. ("Changes</p>
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	<p>tolerant species (fir, spruce) can compete or regenerate well in low light conditions (either under a forest canopy or interspecific competition) and eventually outlive or replace the shade-intolerant species (pine, aspen). In addition to Coates et al. 2013, another paper that explores the interaction of light and soil fertility is Lilles and Astrup 2012. Multiple resource limitation and ontogeny combined: a growth rate comparison of three co-occurring conifers. Can J For Res 42: 99-110.</p>	<p>in species composition and plant productivity are paralleled by changes in N cycle: the less available and less mobile forms of N are, the higher the dependence on symbiotic fungi for nutrition is”).</p> <p>Lines 700-703 have been added, including the reference to Coates et al. 2013 and Messier et al. 1999: “Of course, we acknowledge that soil nutrient availability is not the only factor affecting the rate of growth or forest succession. Light and climatic factors play a fundamental role and must be considered in conjunction with biotic and soil factors, like competition with neighboring plants and availability of adequate substrate for seedling establishment and growth ([196], [189])”</p> <p>See also lines 624-627: “In a recent publication, Coates et al. ([189]) demonstrated that the effect and importance of soil fertility in sub-boreal forests on radial growth of different tree species was context-dependent, affected by competition in species-specific manner and varying with the composition of local neighbourhood of the tree.”</p> <p>The reference to Lilles and Astrup 2012</p>
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		<p>was included at lines 387-390 of the revised manuscript: “In a greenhouse experiment with boreal tree species, Reich et al. [142] measured low rates of N uptake in conifers, while broadleaf species showed high rates of N uptake. In the species tested, the rates of N uptake were related to the relative growth rates and interacted with light availability. At low light, the effect of increased soil fertility was less expressed ([143]).”</p>
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<b><u>Minor</u></b> REVISION comments		
<b><u>Optional/General</u></b> comments		<p>The review article has been subjected to major revisions. Some paragraphs and sentences of the previous version of the manuscript have been removed in order to follow an advice by one of the reviewers and better focus the new manuscript, leaving more space to treat more in detail other issues.</p> <p>Some figures (Figure 3, 4 and 5, of the old manuscript) were also deleted, since the section in which they were included (Section 3, N metabolism and uses) was much reduced in the new version of the manuscript and the text was clear enough without additional figures.</p> <p>The abstract has been partly rewritten accordingly.</p> <p>Some references were added, where needed, following suggestions by reviewers.</p> <p>The modified text is highlighted in yellow.</p> <p>Major phrases / paragraphs deleted, with reference to the lines of the old manuscript:</p> <p>Lines 125-131 (from “through an H<sup>+</sup>-ATPase...” to “NO<sub>3</sub><sup>-</sup> efflux seems to be a passive process, probably through anion</p>



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		<p>channels, but knowledge is still scarce.) Lines 138-142 (from “NH<sub>4</sub><sup>+</sup> nutrition negatively affects the NO<sub>3</sub><sup>-</sup> uptake...” to “NH<sub>4</sub><sup>+</sup> uptake is reduces at high soil and cytosolic NH<sub>4</sub><sup>+</sup> concentrations.”)</p> <p>Lines 234-243 (from “In a long-term experiment with mature Norway spruce trees...” to “...and for root elongation in response to NO<sub>3</sub><sup>-</sup> under NO<sub>3</sub><sup>-</sup> deficiency.”)</p> <p>Lines 290-295 (from “However, the characteristics that probably confers the most competitive...” to “...with values attaining 0.8 t C ha<sup>-1</sup> yr<sup>-1</sup>.”)</p> <p>Lines 304-313 (from “A strategy that could reduce the competitive pressure and enhance conifer nutrition...” to “...in the horizons dominated by ECM fungi.”)</p> <p>Lines 353-379 (all the “3.1 N assimilation” section has been deleted)</p> <p>Lines 382-397 (from “The most common amino acids extracted...” to “...have been observed in Scots pine and white spruce.”)</p> <p>Lines 399-405 (from “Glutamine and</p>
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		<p>glutamate concentrations...” to “...which makes it more sensitive to artificial defoliation of 1-yr old needles.”)</p> <p>Lines 411-413 (from “Seedlings can show both predetermined...” to “...sustaining a second flush of growth.”)</p> <p>Lines 418-424 (from “3.3 N, photosynthesis and growth...” to “...that Rubisco may act as N store during winter.”)</p> <p>Lines 427-432 (from “The slope of the photosynthesis-leaf N relationship...” to “...to the different leaf structure between conifers and angiosperms.”)</p> <p>Lines 434-438 (from “Fertilized Norway spruce showed..” to “...but WUE similar to that of the control”)</p> <p>Lines 451-455 (from “When foliar mass was taken into account...” to “...the biochemical role of proteins in photosynthesis.”)</p> <p>Lines 471-477 (from “N-addition can generate shorter tracheids in wood...” to “...the responses could vary with site</p>
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		<p>because of the different soil and N-availability.”)</p> <p>Lines 478-486 (from “Changes in wood chemistry...” to “...implications on wood decomposition and industrial utilization.”)</p> <p>Lines 507-510 (from “On the basis of a wide European growth dataset...” to “...due to its high C:N ratio.”)</p> <p>Lines 538-540 (from “However, root longevity was lower...” to “...an interaction between temperature and fertilization.”)</p> <p>Lines 685-686 (the sentence “So, it is proposed that late-successional conifers have slow growth because they rely on less available organic N sources.”)</p> <p>Lines 716-721 (from “Moreover, as succession proceeds...” to “...to follow isotopes through the ecosystem and within trees over several years following N addition.”)</p>
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