Response to Reviewers Comments

We thank the reviewers for their thoughtful and supportive comments. We have revised our manuscript in response to their suggestions and hope that this improved manuscript is acceptable for publication in SOIL.

Response to Reviewer # 1:

Comment: Minor comments are: (i) acknowledging the fact that most of these results are for temperate soils

Response: We now acknowledge the focus of this review on temperate soils.

<u>Changes in Manuscript</u>: We have edited the text to "In this review we will first synthesize the current understanding of the nature of the soil N pool and of the factors controlling its long-term storage in temperate region soils."

Comment: (ii) suggested references to incorporate (see specific comments below)

Response: We address this comment in the specific comments section below

Comment: My main comment deals with the implications for policy and management. While the review on soil N tends to cover all possible fates for the soil N, the implications for policy and management section is limited to few (relevant) processes (e.g., saturation, pH). Yet, the author state that "the current understanding of sequestered N [: : :] may also have implications for assessing the effectiveness of ecological restoration practices as well as mitigation strategies for reducing anthropogenic N inputs" (page 14, lines 4–9). I agree, but wonder how we can do that without taking into account the other factors that are known to influence soil N dynamics. Here are a few examples: (1) Invertebrates (e.g. earthworms). The introduction of soil invertebrates can alter SOM stocks (via bioturbation) – and SON dynamics. (2) Agricultural practices. Quid of intensive irrigation (irrigation makes the SOM more hydrophobic, what alters SON behaviors: : :), tillage (depth, period of the year: : :), open-fields (erosion, loss of biodiversity in the soils, etc. alter SON behaviors), N fertilization (quantity and timing –> effects on SON), intensive farming (nitrate...), etc ? The literature on how agricultural practices is particularly abundant, and the mechanisms are extremely well documented. (3) Climate change. Warming. Severe climatic events (drought, floods, etc.). Fires (savanna and forest fires keep increasing). (4) Ramping anthropization of soils. With urban areas in constant increase at the expense of rural areas, policy makers may want to know about how the expansion of the urban areas (i.e., urban soils) may alter SON dynamics and how to counteract/ mitigate such change. (5) Etc. Among the missing factors, many may be managed to optimize soil N and C cycling. As a consequence, I believe that the paper would benefit from a more exhaustive description of the factors that influence N dynamics in soils. These could either be incorporated in the current section 3 (in a paragraph – as for the pH), or separated out from the policy and management implications section. The later option (which I think is clearer) would require a new section that could focus on how these sus-mentioned controlling factors (and the interactions between these factors) influence SON, while the implications for policy and management section (current section 3) could focus on how to take advantage of or counteract their effects.

Response: We appreciate the referee's suggestion for a more exhaustive description of the factors that influence N dynamics in soils. We already discuss fire and have added a discussion of climate change impacts, but as a practical consideration and to keep the scope of the review manageable, we have decided to keep this review to the consideration of temperate ecosystems that are naturally N-limited and that have limited anthropogenic disturbance (beyond the deposition of anthropogenic N). Because of this we have decided not to consider highly anthropogenically influenced soils such as agricultural systems or urban soils, and our decision appears also to be supported by referee 2, who appreciated the focus of the review on natural temperate systems. While we agree that consideration of these factors could be useful in another context, we feel it is beyond the scope of this review and deviates from our intention to focus on natural ecosystems.

<u>Changes in manuscript:</u> Modified the abstract to: "This review examines the factors and mechanisms that influence the long-term sequestration of organic nitrogen in the mineral soil of natural temperate ecosystems". Added paragraph "Climate change and associated increases in floods, droughts and fires will alter N dynamics in soils, but directions and magnitudes are hard to predict since they will depend on the interplay between warming and drying of the soil. Results of studies thus far have been inconsistent and may be highly contingent on site and species specifics (Auyeung et al., 2012). Meta-analysis of warming effects on N dynamics found increased cycling of mineral N, giving credence to studies which have found that warming stimulates N mineralization (Bai et al., 2013). However, the meta-analysis also found only small change to total soil N content, and no change to microbial N immobilization or microbial N was detected, leaving effects on long-term N storage uncertain and in need of further research (Bai et al., 2013)." Additionally to improve readability of section 3 we have subdivided it into three sub-sections: 31.1 …; 3.2….; 3.3 ….

Specific comments:

Comment: Page 10, lines 14–27: suggested references: (1) Kleber et al. 2005, Poorly crystalline mineral phases protect organic matter in acid subsoil horizons, European Journal of Soil Science, December 2005, 56, 717–725, doi: 10.1111/j.1365-2389.2005.00706.x (2) Keiluweit et al 2012, Nano-scale investigation of the association of microbial nitrogen residues with iron (hydr)oxides in a forest soil O-horizon, Geochimica et Cosmochimica Acta 95, 213–226, http://dx.doi.org/10.1016/j.gca.2012.07.001

Page 11: suggested references: (1) Hatton et al 2012, A multi-scale approach to determine accurate elemental and isotopic ratios by nano-scale secondary ion mass spectrometry imaging, Rapid Commun. Mass Spectrom. 2012, 26, 1363–1371, DOI: 10.1002/rcm.6228 (2) Lehman et al 2008, Spatial complexity of soil organic matter forms at nanometre scales, Nature Geoscience, 1, 238-242, doi:10.1038/ngeo155

Response: Thank you for these relevant references, we have included them, with the exception of the Lehman paper as it deals with C and SOM in general, not N.

Comment: Page 13, line 3-5: suggested reference: Hatton et al 2014, Assimilation and accumulation of C by fungi and bacteria attached to soil density fractions, Soil Biology & Biochemistry 79 132-139, http://dx.doi.org/10.1016/j.soilbio.2014.09.013

Response: Thank you for this relevant reference, we have included it.

Comment: Page 14: I suggest emphasizing N and C interactions. Indeed, if we know that N deposition makes a minor contribution to carbon sequestration in temperate forests (Nadelhoffer et al, 1999, Nature 398, 145-148, http://www.nature.com/nature/journal/v398/n6723/abs/398145a0.html), we also know that the fates of N and C in soils are intimately related so that influencing the dynamic of one element necessarily impacts the other (Sollins et al, 2007, Biogeochemistry 85, 1–7, DOI 10.1007/s10533-007-9099-x). As a consequence, assessing practices requires considering both C and N simultaneously.

Response: We have added text to the SOC discussion to highlight the interconnectedness of N and C.

Changes in manuscript: The sentence now reads **"**This is also true for SOC, and forms the basis of the C-saturation model put forth by Six et al. (2002) and observed by Stewart et al. (2007), highlighting the interconnectedness of N and C dynamics in soils."

Technical corrections.

Comment: Page 3, line 18: typo: humifacation –> humification.

Response: Fixed.

Response to Reviewer # 2:

Comment: The authors nicely summarized recent findings on SOM processes in soil but the possible implications for policy and management gained less attention. I would recommend that they come up with an improved concept integrating critical loads and nitrogen saturation/ fluxes in response to the abiotic and biotic properties of natural ecosystems. Using the improved concept it would be valuable to see how current and improved management options differ for a few characteristic natural ecosystems. This would strengthen the focus (and relevance) of this paper.

Response: We appreciate this recommendation and agree that a more in-depth discussion of critical loads and N saturation under this new paradigm would be useful. We have added a discussion to this effect.

Changes in manuscript: Added paragraph "Because N immobilization is now recognized to be driven by ecosystem properties such as edaphic qualities and microbial activity rather than the chemical characteristics of N compounds, more precise and targeted critical loads for N saturation can be developed. Currently, critical loads for N saturation are commonly focused on NO_3^- leaching based on the Aber et al. (1998) framework, which, as discussed above, is not necessarily an indicator of ecosystem N saturation. Using the new paradigm of SON composition and persistence in soils it is possible to identify areas where soil properties and climatic factors are likely to foster lower N processing and decreased stabilization of N. These areas will have a decreased resilience to N saturation and a lower critical load for kinetic N saturation, and may be prioritized for efforts to reduce N deposition."

Comment: The factors microbial processing, adsorption and spatial separation are now discussed separately, and conceptually visualized as an one-dimensional process (figure 1). In reality, there is interaction among the factors throughout the entire soil profile (see also Kaiser & Kalbitz, 2012 and Marin-Spiotta et al, 2014). In consequence of continuous sorption and precipitation as well as of microbial processing, desorption and dissolution the proportions of more recent plant-derived compounds decrease with soil depth while those of microbial metabolites and aged/ microbial processed plant-derived compounds will increase. Soil organic nitrogen changes accordingly. I would recommend the authors to add one section integrating the relevance of the discussed factors across the soil profile (and extending figure 1).

Response: We agree that N is more dynamic than presented, though we believe this simplification is necessary in order to examine the relevant factors in a way which is not overly complicated and that is clear for the reader. We have added a paragraph discussing the dynamic nature of SON and its distribution in the soil profile to highlight the complex nature of SON.

<u>Changes in manuscript:</u> Paragraph added "Once in the soil, SON may cycle between microbial biomass and residues, adsorption and desorption from soil mineral particles, and dissolution and precipitation from the soil solution before it is held in the soil matrix (Knicker, 2011; Marín-Spiotta et al., 2013). While bioturbation can be important, especially in upper soil horizons, model results show that liquid phase transport enables most of the movement of SOM (Braakhekke et al., 2011). The ratio of microbially-derived N compounds compared to plant-derived compounds increases with soil depth, while the C:N ratio and DON concentration decrease (Nannipieri and Paul, 2009; Ros et al., 2009; M. Schmidt et al., 2011). This indicates that plant-derived compounds dominate in the upper soil layers while degraded and processed OM consisting primarily of microbial metabolites migrate in dissolved organic matter (DOM) to lower soil horizons (Kaiser and Kalbitz, 2012)."

Comment: In addition to this, I would recommend to add estimated pool sizes and fluxes (to integrate the processes in section 2) in order to underpin the message of this paper. Anyway, there are numerous qualitative statements in this paper (largest, significant contribution, minor share, decrease quickly, predominantly composed, is likely to vary widely, higher quality, are found to be more important, etc.) that might gain relevance when they were extended with estimated quantitative information. In particular, when the terminology "chemically labile" is used, please give an estimated turnover time since lability is a very diffuse term for 'labile' compounds with turnover rates from seconds to weeks, months or even years.

Response: We agree with the reviewer that more quantitative information would add relevance for the reader and have included estimates for N pool sizes and fluxes. Unfortunately we are not aware of estimates of adsorption or aggregation fluxes and have highlighted the need for quantitative assessment of those fluxes.

We agree that the term 'labile' can be vague and be interpreted as pertaining to any number of different temporal scales. In this paper we use the term labile as a measure of the stability of a compound, or the difficulty of its degradation, rather than as a measure of the length of time of its turnover. We have edited the text to make this distinction clearer.

Changes in manuscript: Pool and flux estimates added to text “In fact, organic compounds can comprise up to 95% of the N in some soils (Rillig et al., 2007; Nannipieri and Paul, 2009; Knicker, 2011), with typical pool sizes of about 725 g N m^{-2}, compared to typical pool sizes of 6 g N m^{-2} for mineral N forms (Sylvia et al., 2005). Amides and amines make up the majority of the organic N pool, and aromatic N compounds, while present, contribute a relatively minor share (Leinweber et al., 2013). Most of this organic N, about 400 Tg yr^{-1} globally (Chapin III et al., 2012), enters the soil as particulate organic matter (POM) through above-ground plant litter and root litter, or in the form of pyrogenic organic matter after fire (Knicker, 2011).”

Text edited to highlight need for better quantification of adsorption “Current views are mixed and studies to better quantify adsorption and desorption fluxes are needed to better evaluate the importance of this mechanism.”

Sentence introducing chemically labile structures edited to “However, empirical evidence of humification has never been found, and advanced chemical analyses of the persistent organic matter pool in soil demonstrated that it is primarily made of chemically labile structures that are easily degraded, with low relative abundance of aromatic groups (Kleber et al., 2011 and references therein).”

Comment: The timing issue might be a relevant item to discuss separately: the focus of this paper is on natural ecosystems with limited N inputs. The relevance of either microbiological or chemical processes - and their controlling factors – vary widely on spatial and temporal scales (as stated in 2.1.) and this certainly has consequences for policy and management.

Response: We agree on the importance of temporal aspects and have discussed this importance in several locations. We have added text to emphasize this importance.

Changes in manuscript: Text edited to “This can lead to rapid losses of SON where soil factors do not favor sequestration, and may lead to losses of N from the ecosystem through nitrification (because NO_3^- is more mobile within soils) and denitrification, especially when C is limiting. “

Text edited to “Conversely, since most N in soils is labile, ecosystems with conditions that favor persistence of N may exhibit signs of elevated N for a long period of time after inputs are reduced as the accumulated N is mined.”

Specific Comments:

Comment: Regarding the discussion on ecosystem N saturation: might it be valuable to link evidence from agricultural studies with those mentioned here? Authors state that “many ecosystems have demonstrated an ability to retain most additional N” and conclude that this state is rarely reached and that there is little evidence of an overall capacity for the retention of N. I fully agree that upscaling is problematic and that not all the processes are well understood, but it seems that I have missed some arguments where the authors conclude that “its importance to a long-term ecosystem N retention is likely to be minimal”.

Response: In this review we have focused on natural ecosystems and have tried to limit the citation of agricultural studies to processes that are important regardless of the degree of disturbance. We are hesitant to rely too heavily on agricultural studies as N-cycling and management options in these systems are vastly different from natural ecosystems.

In this section we are discussing the new concept of capacity saturation and conclude that because there is little to no evidence that capacity saturation is ever reached in most ecosystems (as evidenced by the fact that most ecosystems continue to accumulate N, even at high deposition loads), that capacity saturation is unlikely to be an important consideration for long-term N retention. This is in contrast to kinetic saturation, which we believe does have strong implications for long-term N retention. We have edited the text to make this clearer.

Changes in manuscript: Sentence edited to "Thus, although capacity saturation may occur in small, discrete areas, its importance to long-term ecosystem N retention is likely to be minimal due to its limited occurrence in most systems."

Comment: Are C inputs only decreased due to N inputs (P601): is there also not an increased aboveground (and belowground) biomass production due to elevated N inputs in natural ecosystems? Is it valuable to elaborate on optimum N input levels here? Please, consider not only inorganic N losses here but also the increased (?) potential of DON leaching after microbial processing.

Response: We agree with the author that increased biomass production has also been observed with N inputs and have edited the sentence.

Changes in manuscript: Removed text "(which may decrease themselves due to N input effects)"

Comment: "An increased understanding of the factors governing N storage will improves model input estimates (P603)". Is it possible to come up with a table showing quantitative information (range) of the SMB elements for a few types of natural ecosystems and how the new insights might change these estimates in response to soil or microbiological properties for example?

Response: We have added information about the range of N_i estimates used in SMB equations to illustrate the current uncertainty inherent from such a large range of values. Unfortunately, estimates of how different soil or biological properties affect these values are not available to our knowledge, but would certainly be a valuable area for future research.

Changes in manuscript: Added text for Ni estimates "Estimates of soil N accumulation have ranged from 0.2 kg N ha^{-1} yr^{-1} (Rosen, 1992) to as high as 12 kg N ha^{-1} yr^{-1} (Fenn et al., 2008) for forest ecosystems. This wide range of estimates creates a large uncertainty in the modeled critical load."

Comment: "that unless well protected: : :this N can become available again: : :". This almost suggests that intrinsic biodegradability doesn't exist. I agree that chemical bonding and physical protection are more important than usually thought, but the new conceptual models still include a role for intrinsic recalcitrance. It might be valuable to insights from aquatic studies here (e.g., Marin-Spiotta et al, 2014)

Response: We agree that intrinsic recalcitrance still plays a role in soil N transformation and have fixed the sentence to acknowledge this.

Changes in manuscript: Sentence edited to "Although there exists a gradient of intrinsic recalcitrance within soils, the new understanding that most N is in the form of small, highly labile molecules means that unless well protected from microbial access through protection within the soil matrix, most N can become available again once elevated inputs cease."

Comment: "Edaphic factors: : :may regulate the flux of N : : :" it is possible to come up with concrete recommendations for policy and management here?

Response: We agree that more explicit policy recommendations are useful and have added text to address this.

Changes in manuscript: Paragraph added "Although afforestation of previously disturbed areas will gradually increase N sequestration, the legacy of lost C from these systems means that priority should be given to the protection of old-growth areas that are naturally more resilient to N additions, where years of C accrual has the potential to rapidly sequester much larger amounts of incoming N (Lewis et al., 2014). An improved understanding of the factors important for N persistence can also guide restoration efforts to places where conditions are such that smaller restoration efforts will have a greater impact. For example, soils with a high potential for N persistence but currently exhibiting low microbial activity could be prioritized for revegetation (which would increase moisture levels in soil and promote microbial activity) over areas with less favorable soils characteristics."

Comment: Valuable references Braakhekke et al. (2011) A vertically explicit soil organic matter model, Ecol. Model., 222, 1712–1730, 2011. De Vries et al. (2013) Soil food web properties explain ecosystem services across European land use systems. PNAS. www.pnas.org/cgi/doi/10.1073/pnas.1305198110. Guggenberger & Kaiser (2003) Dissolved organic matter in soil: challenging the paradigm of sorptive preservation. Geoderma 113, 293-310. Kaiser & Kalbitz (2012) Cycling downwards - dissolved organic matter in soils. Soil Biology & Biochemistry. Marin-Spiotta et al. (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. Biogeochemistry. Ros et al. (2009) Extractable and dissolved soil organic nitrogen – a quantitative assessment. Soil Biology & Biochemistry. Wander (2004) Soil Organic Matter Fractions and their relevance to soil function.

Response: We thank the reviewer for these useful references and have utilized them where appropriate.