Referee #1

Comment 1: "Dr. Spohn investigates the relationship between mass-specific microbial respiration, or qCO2, and litter chemistry to understand how nutrient availability affects both mass- specific and total respiration. I like the cross-study approach, and I think it is appropriate for this question."

Answer 1: I would like to thank the reviewer very much for the comments.

Comment 2:"While the author presents some interesting and strong relationships, I think further analysis is needed before the conclusions presented can be made. In particular, I would like to see an analysis that models qCO2 ~ %C + C:N + microbial biomass C + temperature + moisture. Given the correlation between C:N and %C (Table 2) its hard to determine if the relationship is spurious or not. It may just be that higher C:N soils have higher C concentrations, and this drives the increase in qCO2. The author needs to present more detail describing the models that were run, how variables were chosen, etc., which I detail below."

Answer 2: From the results presented in Table 2 and the Figures it can be seen that the correlation between the qCO_2 and the litter layer C:N ratio goes along with a strong negative correlation of the litter layer N concentration and the qCO_2 ($R=0.72$, $p<0.001$), while the correlation between the C concentration and the qCO_2 is not significant ($R=0.26$, $p>0.05$). Thus, it might rather be the N than the C concentration that drives the correlation between the qCO_2 and the C:N ratio. Based on this dataset it is not possible to disentangle whether the correlation between the N concentration and the qCO_2 causes the correlation between the C:N ratio and the qCO_2 or vice versa. It has to be considered that the N concentration (mass N per litter dry mass) is not independent of the C concentration because the C concentration strongly contributes to the dry mass of the litter layer. Plotting the model suggested by the reviewer does not shed more light on this aspect. However, I agree that it is of value model the qCO_2 as a function of all assessed litter properties. Moreover, it makes sense to evaluate the influence of the incubation temperature and the soil water content. Therefore, the following was added to the Material and Method section:

"In order to evaluate the influence of the incubation temperature and the soil water content on the qCO_2, the following linear models were fitted.

$$qCO_2 = a_1 \times C{:}N\ ratio + \varepsilon$$

$$qCO_2 = b_1 \times C{:}N\ ratio + b_2 \times temperature + \varepsilon$$

$$qCO_2 = c_1 \times C{:}N\ ratio + c_2 \times temperature + c_3 \times soil\ water\ content + \varepsilon$$

where a_i, b_i, and c_i are coefficients and ε is the error term. Furthermore, I fitted a linear model with all litter properties and the latitude of the study site of the form

$$qCO_2 = d_1 \times C{:}N\ ratio + d_2 \times temperature + d_3 \times soil\ water\ content + d_4 \times C + d_5 \times N + d_6 \times C_{mic} + d_7 \times N_{mic} + d_8 \times latitude + \varepsilon$$

where d_i are coefficients and ε is the error term."

The following lines were added at the end of the Results.

"The linear regression model of the qCO_2 with the C:N ratio as the only predicting variable had a R^2=0.61 ($p<0.001$). If the incubation temperature was included in the model of the qCO_2 the R^2 increased to R^2=0.72 ($p<0.001$). The R^2 slightly increase further if the soil water content was additionally included as predicting variable (also R^2=0.73, $p<0.001$). If all assessed litter layer properties (C:N ratio, temperature, soil water content, C, N, C_{mic}, N_{mic}) and the latitude were included in the linear model as predicting variables, the R^2 increased to R^2=0.87 ($p<0.001$)."

Accordingly, the following paragraph was added to the Discussion.

"The positive relationship between the incubation temperature and the qCO_2 indicates that the qCO_2 increases with temperature. This influence of the temperature on the qCO_2 is supported by the higher R^2 of the model of the qCO_2 as a function of the C:N ratio and the temperature (R^2=0.72) compared to the model of the qCO_2 as a function of only the C:N ratio (R^2=0.61). The finding that the qCO_2 increases with temperature is in accordance with Xu et al. (2006)."

Comment 3: "Were litter incubations done in the field or lab? If a mix, it would be interesting to know the results when lab/field is included as a covariate."

Answer 3: Only data from laboratory incubations was included as stated in the first line of the second paragraph in the Material and Method section.

Comment 4: "Line 83 "Units were converted to gain microbial biomass C" – I think you mean to obtain. The word gain made me think you were trying to estimate biomass growth for a second."

Answer 4: This verb has been changed as suggested.

Comment 5."Your methods section needs to state the models you ran, which terms were included in each model, and how you decided whether to include or exclude a parameter from a model. Did you include all predictors and then remover them based in AIC scores? Did you include only a subset based on some a-priori reason?"

Answer 5: In the method section, it is clearly explained how the data was processes. "The Pearson's correlation coefficients were calculated, and the significance of the correlation was tested by the Pearson test. All data analysis was conducted in R (R Core Team, 2013)." This is in fact all that was done. In the revised manuscript four linear regression models were added as explained above.

Comment 6: "Line 105 "other statistically significant correlations ... are due to autocorrelation." Did you test for this? How? I think you need to run multiple regression models to get a better handle on this, and check variance inflation factors."

Answer 6: This comment has become obsolete. The term autocorrelation has been changed to "intrinsic dependence of the variables" prior to publication of the manuscript in BGD as suggest earlier by the same reviewer.

Comment 7: "Is there any reason to report Pearson scores rather than R2 values?"

Answer 7: I reported the Pearson's correlation coefficient, which is commonly given as R. In the revised version of the manuscript, additionally the R^2 of the linear regression models are given.

Comment 8: "Lines 125-130- Hessen and Anderson's arguments contradict themselves. "Disposal of C via respiration may need nutrients to maintain the proteins of the respiratory chain." For sure this is true, but it is also true of the alternatives these authors suggest such as storage or building defenses, as those also require N-containing enzymes. If a microbe is already 'fat' with storage compounds, then overflow respiration seems like a reasonable strategy."

Answer 8: There are two arguments by Hessen an Anderson (2008). The first one is that respiration of excess C requires N and that therefore it would be beneficial for microorganisms to dispose of excess C by releasing DOC. In order to be clear about this argument, I will modify the sentence as follows. " It has been argued, first, that for disposing of C via the respiratory chain, N for the proteins of the respiratory chain has to be invested, and therefore it might be more beneficial for microorganisms to dispose of excess C by releasing DOC (Hessen and Anderson, 2008). Second, it has been pointed out that the energy lost by disposing of C could be invested into storage, anti-viral defense or other processes, which increase the fitness of the organism (Hessen and Anderson, 2008; Hessen et al., 2013)." The second argument is discussed in detail in the Discussion, for the Introduction it should be enough to delineate the different arguments. The respective part in the Discussion reads as follows "Yet, it has to be taken into account, first, that the buildup of structural defenses, viral repellents or establishment of symbiosis also requires N, and second, that there are limits to the amounts of C that microbes can store and likely also to the amounts of C microbes can invest into buildup of structural defenses, viral repellents or establishment of symbiosis.".

Comment 9: "An emerging paradigm is that at low C:N there is lower qCO2 because decomposer CUE increases when nutrients are more available (sensu Cotrufo et al. 2013 Global Change Biology). This is consistent with the findings of Bjorn Berg, who you cite. I would strongly recommend you include this in your discussion."

Answer 9: I am aware that the findings about the litter layer stoichiometry and the qCO_2 seem to be in agreement with findings about the microbial carbon use efficiency.

I added the following paragraph to the Discussion: "The findings about the litter layer stoichiometry and the qCO_2 seem to be in agreement with findings about the microbial carbon use efficiency. With increasing C:N ratio microbial carbon use efficiency decreases because the microorganisms do not have enough N to build up as much biomass as the C concentration would allow them (Manzoni et al., 2010; Cotrufo et al., 2013; Sinsabaugh et al., 2013). This seems to agree with the positive correlation between the qCO_2 and the litter C:N ratio. However, it has to be taken into account that the qCO_2 cannot directly be converted into the CUE since the qCO_2 is the ratio of a flux and a pool, and the CUE is the quotient of two fluxes, or in other word since the qCO_2 does not tell how much C was taken up by the microorganisms. Thus, based on the findings presented here no conclusions about microbial carbon use efficiency can be drawn."

Comment 10: “Both in the abstract and in the last paragraph of the discussion the author claims that this relationship may explain increased soil C storage under N deposition. However, most C-stored in soils is of microbial, rather than plant origin (sensu Schmidt et al. 2011 Nature). Given this, can we extend results of leaf-litter studies to make claims about the drivers of soil C storage.”

Answer 10: I claimed that the respiration rate per unit microbial C decreases with decreasing litter C:N ratio. I did not claim that the remaining organic matter is not microbially processed i.e., tuned into microbial biomass and subsequently into dead SOM.

References

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., and Paul, E.: The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter?. Global Change Biol., 19, 988-995, 2913.

Hessen, D. O., and Anderson, T. R.: Excess carbon in aquatic organisms and ecosystems: physiological, ecological, and evolutionary implications, Limnol. Oceanogr., 53, 1685-1696, 2008.

Hessen, D. O., Elser, J. J., Sterner, R. W., and Urabe, J.: Ecological stoichiometry: An elementary approach using basic principles, Limnol. Oceanogr., 58, 2219-2236, 2013.

Manzoni, S., Trofymow, J. A., Jackson, R. B., and Porporato, A.: Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter, Ecol. Monogr., 80, 89-106, 2010.

Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., and Richter, A.: Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling, Ecol. Lett., 16, 930–939, 2013.

Xu, X., Inubushi, K., and Sakamoto, K.: Effect of vegetation and temperature on microbial biomass carbon and metabolic quotients of temperate volcanic forest soils. Geoderma 136, 310-319, 2006.

Referee #2

Comment 1: "Dr. Spohn submitted a manuscript regarding microbial respiratory quotients (qCO2) and litter C:N ratios based on a literature compilation. The manuscript is short, simple, and well-focused on an interesting question relevant to Biogeosciences regarding over- flow metabolism in soil microbes. The literature search resulted in a relatively sparse dataset (14 studies with 48 observations) relative to other literature reviews of qCO2 (e.g., 66 studies and 355 obs, Hartman & Richardson, 2013). However this is to be expected, as Dr. Spohn's manuscript focuses on qCO2 in litter, rather than soil. This is an appropriate choice for this manuscript, as the high C:N ratio of litter relative to microbial biomass is particularly relevant to the subject of overflow metabolism. I enjoyed reading this manuscript, and the results are clear and compelling. However I have some concerns and suggestions that I hope will serve to improve the manuscript."

Answer 1: I would like to thank the reviewer very much for the constructive comments.

Comment 2: "Major concerns:

(1) The author introduces overflow metabolism as a controversial subject of current debate; however the existence of overflow metabolism in some organisms is indisputable and has been the subject of several decades of research. Overflow metabolism is clearly supported by molecular biology work in plant mitochondria, as the alternative oxidase and uncoupler proteins allow for the oxidation of organic molecules into CO2 without a corresponding production of ATP (Atkin et al., 2005; Plaxton & Podesta, 2006). There is also a well-developed literature on overflow metabolism in bacteria, particularly E. coli, although the molecular mechanisms seem to be different (e.g., Vemuri et al., 2006). While I understand that the molecular mechanisms are not fully understood in the complex community of organisms that decompose litter, I suggest that the author briefly acknowledge this literature as support for the general concept of overflow metabolism."

Answer 2: It's true that the manuscript reads as if there was still discussion about the existence of the process itself, and not only about its relevance in ecosystems. I will add a sentence on overflow respiration in microorganisms referring to the mentioned study by Vermuri et al. (2006) and to two review papers on the subject (Russell and Cook, 1995; Teixeira de Mattos and Neijssel, 1997). Moreover, I will state that there is debate about the relevance of this process in ecosystems, but not about the existence of the process in microorganisms itself.

Comment 3: "(2) Line 53-55. There is little reason to expect overflow metabolism to be forest-specific, so why limit the data compilation to the forest literature? Consider broadening the analysis to include studies regarding litter decomposition in other systems (e.g., grasslands) and residue decomposition in crop systems."

Answer 3: I did not restrict the analysis to forest soil litter layers. In fact, some of the data come from the soil litter layer of Coco plantations and of a heathland (see Table 1). I did not find more data from ecosystems other than forest that met the criteria of the literature search. Since this analysis deals with soil litter layers, studies that measured the qCO_2 on plant detritus were not considered (and I am also not aware of any study that measured the qCO_2 on plant detritus).

Comment 4: “(3) Lines 119-130. This reads like the author is pursuing to discredit the notion of overflow metabolism, when the results clearly support it. I suggest the author clearly state that the results were consistent with overflow metabolism in the decomposition of forest litter, possibly in the first and/or last paragraphs of the discussion section. Furthermore, I am unconvinced by the argument on line 127 that “... microorganisms may use C that is in surplus to their demands of somatic growth for promoting their fitness by C storage, buildup of structural defenses, viral repellents or establishment of symbiosis.” The additional processes listed by the author are not infinite C sinks. Consider the case that the microorganisms have already satisfied the C demands of structural defences, viral repellents, etc; what should they do with the “extra” C in this case? The concept of satisfied C demands need not be confined to somatic growth.”

Answer 4: In the discussion section of the manuscript, three possible explanations for the main finding are critically discussed. All three discussed mechanisms could potentially explain the observed relationships. In order to be more explicit, I will add the following sentence at the end of the discussion of the three possible explanations. “All three mechanisms can explain the observed relationship between the qCO_2 and the soil litter layer C:N ratio; and based on the data presented here it cannot be concluded which of the three mechanisms is most relevant to the observed relationship.”

The reviewer is right in saying that there are limits to the amounts of C that can be stored by microorganisms or invested into buildup of structural defenses, viral repellents or establishment of symbiosis. Though not infinite, the amounts of C that microorganisms can invest into establishment of symbiosis, the release of low weight molecular substances or communication are likely very large. I will add a sentence, stating that there are limits to the amounts of C that microbes can store and likely also to the amounts of C microbes can invest into buildup of structural defenses, viral repellents or establishment of symbiosis. The size of these limits, i.e. the amounts of C that microbes can invest into other processes than somatic growth, remain to be explored.

Comment 5: “Minor concerns:

(1) The authors report a three-part analysis showing that (1) qCO2 was positively correlated with litter C:N, (2) basal respiration was positively correlated with litter C:N, and (3) microbial biomass was not correlated with litter C:N. This exploration of the data was very well done. The reader may be able to see this most clearly if point #3 was demonstrated with a figure. Please consider a 3-panel figure with qCO2, basal respiration, and microbial biomass all plotted in relation to litter C:N.”

Answer 5: I considered adding another figure, showing that there’s no correlation between the soil C:N ratio and the microbial C:N ratio. I decided not to do this because it is common practice to only show correlations, but not the absence of correlations in figures. The correlation coefficients are given in Table 2 anyway.

Comment 6: “(2) lines 106, 113- tense change; consistently use the past tense. It is common practice to discuss previously published literature in the present tense to recognize the current

relevance of the established research. However it is more appropriate to discuss the current manuscript in the past tense."

Answer 6: Yes, I will correct this.

Comment 7: "(3) Line 137. "Adapt" has a specific biological meaning that is not appropriate here"

Answer 7: That's true. I will replace "adapt" by "adjust".

References

Russell, J. B., and Cook, G. M.: Energetics of bacterial growth: balance of anabolic and catabolic reactions, Microbiol. Rev., 59, 48-62, 1995.

Teixeira de Mattos, M., and Neijssel, O.M.: Bioenergetic consequences of microbial adaptation to low-nutrient environments, J. Biotech., 59, 117-126, 1997.

Vemuri, G. N., Altman, E., Sangurdekar, D. P., Khodursky, A. B., and Eiteman, M. A.: Overflow metabolism in Escherichia coli during steady-state growth: transcriptional regulation and effect of the redox ratio, Appl. Environ. Microbiol., 72, 3653-3661, 2006.

Microbial respiration per unit microbial biomass depends on ~~soil~~ litter layer carbon-to-nitrogen ratio

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Abstract

Soil microbial respiration is a central process in the terrestrial carbon (C) cycle. In this study, I tested the effect of the carbon-to-nitrogen (C:N) ratio of soil litter layers on microbial respiration in absolute terms and per unit microbial biomass C. For this purpose, a global dataset on microbial respiration per unit microbial biomass C – termed the metabolic quotient (qCO_2) – was compiled form literature data. It was found that the qCO_2 in the soil litter layers was positively correlated with the litter C:N ratio and was negatively related with the litter nitrogen (N) concentration. The positive relation between the qCO_2 and the litter C:N ratio resulted from an increase in respiration with the C:N ratio in combination with no significant effect of the litter C:N ratio on the soil microbial biomass C concentration. The results suggest that soil microorganisms respire more C both in absolute terms and per unit microbial biomass C when decomposing N-poor substrate. The reasons for the observed relationship between the qCO_2 and the litter layer C:N ratio could be microbial N mining, overflow respiration or the inhibition of oxidative enzymes at high N concentrations. In conclusion, the results show that the qCO_2 increases with the litter layer C:N ratio. Thus, the findings indicate that atmospheric N deposition, leading to decreased litter C:N ratios, might decrease microbial respiration in soils.

1 Introduction

Large amounts of organic carbon (C) are transformed, stored and respired by microorganisms in soil. Hence, gaining insight into the factors controlling the respiration rate per unit soil microbial biomass is crucial to understand the terrestrial C cycle. The respiration rate per unit microbial biomass C – termed the metabolic quotient (qCO_2) – is as a measure for the ecophysiological status of soil microorganisms (Anderson and Domsch, 1993). Although a large number of studies on the qCO_2 has been published (reviewed by Brookes, 1995; Bastida et al., 2008; Anderson and Domsch, 2010), little is known about how the qCO_2 is affected by soil C:N:P stoichiometry.

The soil microbial biomass shows a relatively well constrained stoichiometry similarly to the Redfield ratio found for planktonic biomass (Redfield, 1934). Although the stoichiometry of individual phylogenetic groups may vary, the molar C:N ratio of the soil microbial biomass at a global scale converges towards 6-8 (Cleveland and Liptzin, 2007; Xu et al., 2013). The C:N ratio of soil litter layers is in the range of 12-80 (Berg and McClaugherty, 2003). Thus, microorganisms decomposing litter with a high C:N ratio are confronted with a surplus of C in relation to N. Compared to other ecosystems, microorganisms in forests face extreme substrate imbalances since the C:N ratios of woody plants are extremely high compared to the microbial biomass C:N ratio. While, for example, in phytoplankton and magroalgae the C:N ratio amounts to approximately 10, woody plants have a C:N ratio of up to 400 (Cebrian, 1999; Sterner and Elser, 2002).

When growing on N-poor substrate, microorganisms have not enough N to build up as much biomass as the C concentration would allow. Thus, it has been argued that microorganisms can dispose of C via overflow respiration as CO_2 to make the substrate meet their nutritional demands (Manzoni et al., 2008, 2010; Sinsabaugh et al., 2013). Overflow respiration, i.e., ~~is thought to be~~ respiration without the production of energy, has been shown to occur in several microbial species in laboratory incubations (Russell and Cooks 1995; Teixeira de Mattos and Neijssel, 1997; Vemuri et al., 2006). The ~~concept~~relevance ~~-~~ of microbial overflow respiration in ecosystems has recently been ~~criticized~~ questioned by several studies. It has been argued, first, that for disposing of C via the respiratory chain, N for the proteins of the respiratory chain has to be invested, and~~,~~ therefore it might be more beneficial for microorganisms to dispose of excess C by releasing DOC (Hessen and Anderson, 2008). S~~s~~econd, it has been pointed out that the energy lost by disposing of C could be invested into storage, anti-viral defense or other processes, which increase the fitness of the organism (Hessen and Anderson, 2008; Hessen et

al., 2013). Hence, while overflow respiration has been shown to occur in laboratory incubations and seems to be likely from a stoichiometric perspective ~~of stoichiometric models~~, the ~~existence~~ relevance of this process in ecosystems is still under discussion.

The objective of this study was to use data of published studies on the qCO_2 in soil litter layers to learn about how litter C:N stoichiometry affects the respiration rate per unit decomposer biomass. Following stoichiometric theory, I tested the hypothesis that the qCO_2 increases with litter C:N ratio and decreases with litter N concentration. For this purpose, data from literature on the qCO_2 in soil litter layers and on litter layer properties was compiled.

2 Material and methods

Literature searches were conducted using Google Scholar, Web of Science, and Scopus in November and December 2013. I searched for the word "metabolic quotient" in combination with the following terms "litter decomposition", "litter layer", "leaf decomposition", "needle decomposition", "microbial activity", "forest floor", "microbial respiration", "tropical forest", " temperate forest", "boreal forest", "mediterranean forest", "plantation".

Based on the literature search, I selected studies that reported the qCO_2 measured in laboratory incubations on litter collected from the soil litter layer of forests, tree and palm plantations, and heathlands. Studies that mixed litter with mineral soil were excluded because it is assumed that stabilization of the soil organic matter by sorption and aggregation possibly obscures relations between element concentrations and the qCO_2. If results for different treatments were reported, only the data for the control treatment were extracted. If time series were reported, I only extracted the first data point of the series in order to avoid pseudo-replication. In order to prevent confounding results due to different methods, the following criteria were applied for data selection. The qCO_2 had to be reported in unambiguous units as the ~~rate of C mineralization~~respiration rate per unit of microbial biomass C. Basal respiration had to be determined during incubations based on CO_2 measurements by gas chromatography or titration (but not, for example, O_2 consumption), and the microbial biomass C had to be determined by the fumigation-extraction method. Additionally, the studies had to report either the C:N ratio of the litter or both the C and N concentration. Besides the metabolic quotient, microbial biomass C (C_{mic}), basal respiration, and the C:N ratio of the litter, the following parameters were collected if reported in the studies: latitude and mean annual temperature of the study site, classification of the litter layer, litter pH, plant species from which the litter was derived,

microbial biomass N (N_{mic}), litter P, microbial biomass P, and temperature and soil water ~~holding~~ capacity at which the respiration measurement had been performed. In case data was reported in the form of graphs, numbers were extracted using the open-source software DataThief (Tummers, 2006).

Units were converted to obtain~~gain~~ microbial biomass C in mg (g litter)$^{-1}$, basal respiration in µg CO_2-C (g litter-C)$^{-1}$ h^{-1}, qCO_2 in µg CO_2-C (mg microbial-C)$^{-1}$ h^{-1}, and the C:N ratio in mol mol^{-1}. For all analyses including latitude, only the degree of latitude was considered, but no differentiation between Southern and Northern hemisphere was made. The Pearson's correlation coefficients were calculated, and the significance of the correlation was tested by the Pearson test. In order to evaluate the influence of the incubation temperature and the soil water content on the qCO_2, the following linear regression models were fitted.

$$qCO_2 = a_1 \times C{:}N\ ratio + \varepsilon$$

$$qCO_2 = b_1 \times C{:}N\ ratio + b_2 \times temperature + \varepsilon$$

$$qCO_2 = c_1 \times C{:}N\ ratio + c_2 \times temperature + c_3 \times soil\ water\ content + \varepsilon$$

where a_i, b_i, and c_i are coefficients and ε is the error term. Furthermore, I fitted a linear model with all litter properties and the latitude of the study site of the form

$$qCO_2 = d_1 \times C{:}N\ ratio + d_2 \times temperature + d_3 \times soil\ water\ content + d_4 \times C + d_5 \times N + d_6 \times C_{mic} + d_7 \times N_{mic} + d_8 \times latitude + \varepsilon$$

where d_i are coefficients and ε is the error term. All data analysis was conducted in R (R Core Team, 2013).

3 Results

Fourteen studies were found that met the above-mentioned criteria, resulting in 48 observations. The studies covered the tropical, temperate, and boreal climate zone, and included data on the qCO_2 measured on litter derived from seven tree genera. Additionally, two studies reported data on litter of mixed forests with non-characterized species composition, and two studies reported results on litter derived from a palm and legumes and a forb (Table 1).

The qCO_2 was positively related to the C:N ratio of the litter (slope=0.14, r~~R~~=0.78, p<0.001, Fig. 1) and negatively to the litter N concentration (slope=0.30, r~~R~~=-0.72, p<0.001, Fig. 2). The

positive relation between litter C:N ratio and qCO_2 resulted from a positive relation between respiration and the C:N ratio (slope=1.47, r~~R~~=0.71, p<0.001, Fig. 3), and no effect of the litter C:N ratio on the microbial biomass C concentration (r~~R~~=0.16, p>0.05, Table 2). The incubation temperatures, at which the respiration rates had been determined~~,~~ ranged from 14 to 25°C. ~~Some of the variation in t~~The qCO_2 was ~~due to the different incubation temperatures and the positive correlation between~~positively correlated with the incubation temperature ~~and qCO_2~~ (slope=0.25, r~~R~~=0.55, p<0.001, Table 2). Moreover, the latitude was negatively related with the litter N concentration (r~~R~~=-0.51, p<0.001, Table 2). Other statistically significant correlations, such as between respiration rate and qCO_2, and N concentration and C:N ratio (Table 2), ~~are~~ were due to the intrinsic dependence of the variables~~autocorrelation~~. No significant relation between the litter C:N ratio and the microbial C:N ratio was found (r~~R~~=0.11, p>0.05, Table 2). Unfortunately, only very few studies reported litter P or microbial P concentrations, ~~making~~ rendering the inclusion of these parameters into the analysis impossible.

The linear regression model of the qCO_2 with the C:N ratio as the only predicting variable had a R^2=0.61 (p<0.001). If the incubation temperature was included in the model of the qCO_2 the R^2 increased to R^2=0.72 (p<0.001). The R^2 slightly increase further if the soil water content was additionally included as predicting variable (also R^2=0.73, p<0.001). If all assessed litter layer properties (C:N ratio, temperature, soil water content, C, N, C_{mic}, N_{mic}) and the latitude were included in the linear model as predicting variables, the R^2 increased to R^2=0.87 (p<0.001).

4 Discussion

Here it was found that soil microbial respiration rate both in absolute terms and per unit microbial biomass ~~is~~ was positively correlated with the soil litter C:N ratio. The findings are in accordance with previous studies that reported a positive correlation between litter C:N ratio and respiration (Othonen, 1994; Gödde et al., 2002; Michel and Matzner, 2002), and a negative relation between respiration and available N (Craine et al., 2007). The findings also agree with results from litterbag studies on litter decomposition in relation to litter C:N ratio (Berg and Matzner, 1997; Berg and McClaugherty, 2003). Moreover, the findings go in line with a positive correlation between the qCO_2 and the soil C-to-nutrient ratios in beech, spruce and mixed forests found recently (Spohn and Chodak, 2015).

There are at least three explanations for the observed relationships ~~Several explanations for this negative relationship between respiration and C:N ratio have been proposed~~. A first explanation

might be that microorganisms mine litter for N, i.e., they burn readily available C in order to gain energy to acquire N from more recalcitrant forms of organic matter (Craine et al., 2007) or in order to have physical access to the N incorporated in organic compounds. However, it can be questioned whether microorganisms that suffer from N limitation can afford to invest N into the production of exoenzymes and release them to acquire C, especially in N poor soils where the pay-off in terms of N is very small. A second explanation ~~is based on stoichiometry theory. It states that excess C is burned through~~might be `overflow respiration´, which means that microorganisms uncouple respiration from energy production and only respire C to dispose it of (Russel and Cook et al., 1995; Manzoni et al., 2008, 2010). Overflow respiration has been observed in many microbial species in lab incubations (Russell and Cooks 1995; Teixeira de Mattos and Neijssel, 1997). However, ~~this argument~~the relevance of microbial overflow respiration in ecosystems has been ~~criticized~~ questioned for two reasons (Hessen and Anderson, 2008). First, the disposal of C via respiration requires N to maintain the proteins of the respiratory chain, and thus it would be more beneficial for microorganisms to dispose of excess C by releasing DOC (Hessen and Anderson, 2008). Second, microorganisms may use C that is in surplus to their demands of somatic growth for promoting their fitness by C storage, buildup of structural defenses, viral repellents or establishment of symbiosis. Yet, it has to be taken into account, first, that the buildup of structural defenses, viral repellents or establishment of symbiosis also requires N, and second, that there are limits to the amounts of C that microbes can store and likely also to the amounts of C microbes can invest into buildup of structural defenses, viral repellents or establishment of symbiosis. A third explanation for decreased respiration at low litter C:N ratios could be that the activity of oxidative enzymes involved in the degradation of aromatic compounds decreases with N concentration (Carreiro et al., 2000; Saya-Cork et al., 2002; Michel and Matzner, 2003; Gallo et al., 2004). Decreased lignolytic activity might decrease microbial respiration in litter with low C:N ratios (Carreiro et al., 2000; Eiland et al., 2001; Saya-Cork et al., 2002). All three mechanisms – N mining, overflow respiration, and enzyme inhibition – could explain the observed relationship between the qCO_2 and the litter layer C:N ratio; and based on the data presented here it cannot be concluded which of the three mechanisms is most relevant to the observed relationships.

The positive relationship between the incubation temperature and the qCO_2 indicates that the qCO_2 increases with temperature. This influence of the temperature on the qCO_2 is supported by the higher R^2 of the model of the qCO_2 as a function of the C:N ratio and temperature (R^2=0.72) compared to the model of the qCO_2 as a function of only the C:N ratio (R^2=0.61). The finding that the qCO_2 increased with temperature is in accordance with Xu et al. (2006).

The findings about the litter layer stoichiometry and the qCO_2 seem to be in agreement with findings about the microbial carbon use efficiency. With increasing litter C:N ratio, microbial carbon use efficiency decreases because the microorganisms do not have enough N to build up as much biomass as the C concentration would allow them (Manzoni et al., 2010; Cotrufo et al., 2013; Sinsabaugh et al., 2013). This seems to agree with the positive correlation between the qCO_2 and the litter C:N ratio. However, it has to be taken into account that the qCO_2 cannot directly be converted into the CUE since the qCO_2 is the ratio of a flux and a pool, and the CUE is a ratio of two fluxes, or in other word since the qCO_2 does not tell how much C was taken up by the microorganisms. Thus, based on the findings presented here no conclusions about microbial carbon use efficiency can be drawn.

One further way in which microorganisms can react to imbalanced substrate stoichiometry, is to gradually ~~adapt~~ adjust the microbial biomass stoichiometry to the substrate as recently shown for microorganisms in tropical litter (Fanin et al., 2013). However, in this study, I did not find a significant relation between the litter C:N ratio and the microbial C:N ratio, indicating that the microbial community did not adapt its biomass composition to the litter layer stoichiometry.

There are several implication soft the relationships found here. The positive corelation between qCO_2 and litter C:N ratio resulted from an increase in~~–~~ respiration with the C:N ratio in combination with no significant effect of the litter C:N ratio on the soil microbial biomass C concentration. The findings of this study indicate that atmospheric N deposition, leading to decreased litter C:N ratios, might decrease microbial respiration in soil litter layers both in absolute terms and per unit microbial biomass. This is in accordance with studies ~~reporting~~ that reported that long-term N deposition and fertilization, resulting in decreased~~s in~~ plant litter C:N ratios, increased soil C sequestration in forests (Magnani et al., 2007; Pregitzer et al., 2008; Janssens et al., 2010). Pregitzer et al. (2008) and Janssens et al. (2010) found that the major reason for the positive effect of N deposition on C sequestration is reduced respiration with decreasing soil C:N ratio. The present~~is~~ study suggests that this reduction in respiration rates is not due to a lower microbial biomass concentration, but due to a reduced respiration rate per unit microbial biomass. Another implication of the results presented here concerns soil and ecosystem models. In these models, the proportion of C emitted per unit decomposer biomass is usually thought to be constant (Manzoni and Porporato, 2009). However, here it was shown that it is highly dependent on the soil litter layer C:N ratio.

5 Conclusions

This analysis of literature data shows that microbial respiration per unit microbial biomass in soil litter layers increases with the litter C:N ratio, highlighting the importance of soil stoichiometry for microbial mineralization processes. The findings indicate that atmospheric N deposition, leading to decreased litter C:N ratios, might decrease microbial respiration in soils.

Appendix A

A list of the publications used for data extraction can be found in the supplementary material.

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Table 1. References considered in the analysis together with the latitude of the study site, the plant genus from which the litter was derived and the number of data points ~~gained~~ obtained from each reference. A detailed list of the publications, from which data was extracted is given in the supplementary material.

Reference	**Latitude**	**Plant**	**Data points**
Chang and Trofymow, 1996	50°N	*Cedrus*	3
Chapman et al., 2003	57°N	*Pinus*	1
Dinesh et al., 2006	10°S	*Cocos* & Legumes	10
Fisk and Fahey, 2001	44°N	*Fagus & Betula*	1
Karneva and Smolander, 2007	66°N	*Picea, Pinus, Betula*	8
van Meeteren et al., 2007	52°N	Forbs	1
Ndaw et al., 2009	21°S	Various broadleaf trees, *Eucalyptus*	4
Pietikainen and Fritze, 1996	65°N	*Picea*	3
Ross & Sparling, 1993	36°S	*Pinus*	4
Ross and Tate, 1993	36°S	*Fagus*	2
Ross et al., 1996	43°S	*Fagus*	2
Ross et al., 1999a	38°S	Various trees, *Pinus*	4
Ross et al., 1999b	61°N, 42°S, 40°S, 36°S	*Pinus*	4
Schimel et al., 1999	64°N	*Betula*	1

Table 2. Pearson's ~~Spearman's~~ correlation coefficient of the latitude of the study site, the pH_{H2O} of the soil litter layer, the C and N concentration and the C:N ratio of the soil litter layer, the microbial biomass C and N concentration (C_{mic} and N_{mic}), the microbial biomass C:N ratio, the incubation temperature at which the respiration rate was determined (Temp), the respiration rate (Resp), and the metabolic quotient (qCO_2). *, **, *** denote levels of significance at p<0.05, 0.01 and 0.001.

	Latitude	**pH_{H2O}**	**C**	**N**	**C:N**	**C_{mic}**	**N_{mic}**	**C_{mic}:N_{mic}**	**Temp**	**Resp**	**qCO_2**
Latitude											
pH_{H2O}	-0.39*										
C	0.52***	-0.16									
N	-0.51***	-0.14	0.00								
C:N	0.38**	0.17	0.51*	-0.81***							
C_{mic}	0.22	-0.12	0.24	-0.01	0.16						
N_{mic}	-0.01	0.25	0.13	-0.20	0.22	0.08					
C_{mic}:N_{mic}	0.04	-0.07	0.18	0.00	0.11	0.54***	-0.39*				
Temp	-0.42**	0.39*	0.17	-0.38*	0.30*	-0.06	0.40**	0.03			
Resp	0.17	0.19	0.35*	-0.56***	0.71***	0.52***	0.38*	0.07	0.33*		
qCO_2	0.13	0.36*	0.26	-0.72***	0.78***	0.01	0.22	0.05	0.55***	0.64***	

575 **Figure captions**

576 Figure 1. Correlation between the metabolic quotient (qCO_2) and the molar carbon-to-nitrogen
577 ratio (C:N) of the soil litter layer

578

579 Figure 2. Correlation between the metabolic quotient (qCO_2) and the soil litter layer nitrogen
580 (N) concentration

581

582 Figure 3. Correlation between the basal respiration rate and the molar carbon-to-nitrogen ratio
583 (C:N) of the soil litter layer