LEONARDUS VERGÜTZ

STUDYING THE SOIL COMPARTMENT OF THE GLOBAL CARBON CYCLE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título *Doctor Scientiae*.

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APROVADA: 12 de agosto de 2011

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(Coorientador)

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Prof. Roberto Ferreira de Novais
(Orientador)

Dedico esta tese aos meus pais ***ILMO*** e ***NELI*** e à minha esposa ***TERESA CRISTINA***

"Education is the most powerful weapon which you can use to change the world"

Nelson Mandela

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Finalmente, a todos que direta ou indiretamente me apoiaram e incentivaram ao longo do caminho;

MUITO OBRIGADO!!!

BIOGRAFIA

Leonardus Vergütz, filho de Ilmo Vergütz e Neli de Jesus Vergütz, nascido em 23 de março de 1982, em Patos de Minas, MG.

No ano de 2000 mudou-se para Viçosa, MG, onde iniciou o curso de Agronomia pela Universidade Federal de Viçosa.

Em 2005 obteve o título de bacharel e em agosto desse mesmo ano deu início aos estudos em nível de mestrado pelo programa de pós-graduação em Solos e Nutrição de Plantas dessa mesma universidade, desenvolvendo trabalhos sobre o impacto do cultivo de eucalipto nos estoques de C orgânico dos solos.

Em agosto de 2007 defendeu sua dissertação de mestrado, dando início ao curso de doutorado em Solos e Nutrição de Plantas nesse mesmo mês. Durante seu doutoramento dedicou esforços para melhorar o conhecimento a respeito do ciclo do C nos solos, realizando estudos quantitativos e qualitativos que incluem o estudo calorimétrico das substâncias húmicas.

Em setembro de 2009 mudou-se para Raleigh, NC, USA, onde iniciou o programa de doutorado sanduíche no *Department of Biology* da *Duke University* sob orientação do professor Robert B. Jackson. Permaneceu na *Duke University* até dezembro de 2010, tendo desenvolvido trabalho de meta-análise sobre a concentração e reabsorção global de nutrientes em folhas de plantas terrestres.

Defendeu sua tese de doutorado em 12 de agosto de 2011.

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VERGÜTZ, Leonardus, D.Sc., Universidade Federal de Viçosa, agosto de 2011. **Studying the soil compartment of the global carbon cycle.** Orientador: Roberto Ferreira de Novais. Coorientador: Ivo Ribeiro da Silva.

Mudanças climáticas globais causadas pelo aumento da concentração de CO_2 atmosférico tem estimulado uma melhor compreensão do ciclo global do C. Os solos representam um dos maiores compartimentos de C na Terra, contendo mais C que na biomassa vegetal e na atmosfera. Além disso, o C orgânico do solo (COS) também é um compartimento bastante dinâmico. Portanto, compreender o papel do COS para o ciclo global do C e como ele é afetado por diferentes condições edafoclimáticas se faz extremamente importante, especialmente para países como o Brasil, onde a maioria das emissões de gases causadores do efeito estufa são provenientes de mudanças de uso do solo e atividades agropecuárias. O objetivo geral deste trabalho foi estudar quantitativa e qualitativamente o COS, visando a melhor compreensão dos fatores que levam à sua estabilização. Para isso foram investigados três estágios que afetam a ciclagem do C nos solos, sendo eles: as concentrações e eficiências de reabsorção global do C e de nutrientes em plantas terrestres (Chapter 1), o estudo da estrutura e do comportamento dos ácidos húmicos pela técnica de titulação calorimétrica isotérmica (microcalorimetria) (Chapter 2) e a influência das argilas e de condições climáticas nos estoques de COS no Brasil (Chapter 3). Do primeiro estudo (Chapter 1) resultaram novas estimativas globais de concentrações e de eficiências de reabsorção de nutrientes. Neste trabalho foi incorporada a perda de massa que ocorre nas folhas durante a senescência, fato esse até então desconsiderado em estimativas globais e responsável pela subestimação da real reabsorção de C e nutrientes. Em geral, as folhas verdes perdem 24% de sua massa seca durante o processo de senescência. Levando essa perda em consideração as reabsorções de C, N, P, K, Ca e Mg foram de 23,2; 62,1; 64,9; 70,1; 10,9 e 28,6%, respectivamente. Esses resultados devem levar à melhoria de modelos que explicitamente representam a ciclagem de C e nutrientes, além de permitir que os pesquisadores que trabalham com modelagem associem os ciclos de outros nutrientes ao nível de grupos de plantas e ecossistemas. No segundo trabalho (Chapter 2) empregou-se uma técnica relativamente nova para o estudo da estrutura e comportamento dos ácidos húmicos. A partir desta análise foi possível demonstrar que existe uma estrutura básica (monômeros) dos ácidos húmicos e que, ao contrário do que o usual modelo supramolecular prega, essa estrutura básica não é formada por unidades simples fracamente unidas passíveis de sofrer divisões ou mudanças conformacionais durante interação. Adicionalmente, esses monômeros se mostraram similares no ambiente, não importando sua fonte, composição ou processo de estabilização. Finalmente, no último trabalho (Chapter 3) foram analizados os estoques de COS em áreas de vegetação nativa, eucalipto e pastagem de três principais biomas brasileiros. Em geral, os estoques de COS na camada de 0-100 cm para as áreas de vegetação nativa, eucalipto e pastagem de todos os biomas em conjunto foram 137,3; 127,9 e 127,1 t ha^{-1}, respectivamente. Este tipo de trabalho em escala regional é necessário para melhorar as estimativas globais de COS. Neste trabalho foi mostrado que partículas de argila de solos argilosos, especialmente nas camadas mais profundas, ainda não se encontram saturadas com COS, indicando que existe a possibilidade de aumento do sequestro de C em solos brasileiros. Adicionalmente, o aumento da mineralização do COS após mudança de uso do solo se correlacionou positivamente com o aumento da temperatura, indicando que a oxidação do COS será ainda maior em um cenário de mudanças climáticas globais. Em geral, este trabalho contribui de diferentes maneiras para a melhor compreensão do compartimento solo no ciclo global do C.

ABSTRACT

VERGÜTZ, Leonardus, D.Sc., Universidade Federal de Viçosa, August, 2011. **Studying the soil compartment of the global carbon cycle.** Advisor: Roberto Ferreira de Novais. Co-advisor: Ivo Ribeiro da Silva.

Climate changes caused by increased atmospheric concentration of CO_2 have stimulated a better understanding of the global C cycle. Soils represent one of the largest compartments of C on Earth, with higher C content than plant biomass and atmosphere. Besides its size, soil organic C (SOC) is also a very dynamic compartment. Thus, comprehend the role of SOC and how it is affected by edaphoclimatic conditions is of great importance, especially to Brazil, once the majority of its greenhouse gases emissions come from land use change and agriculture. The general objective of this work was to study SOC quantitative and qualitatively, in order to improve the knowledge about the factors that lead to its stabilization. To this end we attempted to investigate three different stages of it, namely: global resorption efficiencies and concentrations of C and nutrients in terrestrial plants (Chapter 1), the evaluation of humic acid’s structure and behavior through isothermal titration calorimetry (microcalorimetry) (Chapter 2), and the role of clay and climatic conditions onto soil organic carbon in Brazilian soils (Chapter 3). On the first study (Chapter 1) we provided new global estimates for nutrient concentrations and resorption efficiencies. In this work we took into account the leaf mass loss occurring during senescence, which to our knowledge has been neglected so far in global estimates and leads to an underestimation of the real resorption. In general, leaves lose up to 24% of its dry mass during senescence. Taken it into account, C, N, P, K, Ca, and Mg resorptions were, on average, 23.2, 62.1, 64.9, 70.1, 10.9, and 28.6%, respectively. It should improve models that explicitly represent the cycling of C and nutrients, and also allow the modeling community to represent more explicitly the coupling of other nutrient cycles within plants and ecosystems. On Chapter 2 we employed a fairly recent technique and showed new insights on humic acid’s structure and behavior. The results demonstrated that indeed humic acids show a basic structure (monomers) and that, contrary to what the well accepted supramolecular model preaches, humic acid basic structure is not loosely held nor does it show any division or conformational change when undergoing an interaction. We also demonstrated here that these monomers are kept virtually the same regarding differences on its sources, composition, and humification processes. Lastly, on Chapter 3 we showed data on SOC stocks under native vegetation, eucalyptus, and pasture within three major Brazilian biomes. In general, SOC stocks at the first soil meter for native vegetation, eucalyptus, and pasture areas were 137.3, 127.9, and 127.1 t ha^{-1}, respectively. This kind of regional scale work is necessary to improve overall estimates of SOC stocks globally. Besides that, our results showed that clay particles in clayey soils, especially in deeper layers, are still not saturated with SOC, showing that there is still room to sequester C deep in Brazilian soils, helping offset CO_2 emissions. Still on the light of global climate changes, our results showed that increasingly temperature is associated with an increase on SOC mineralization after land use change, making it more difficult to remediate. Overall, our work contributes in different ways to a better understanding of the soil compartment of the global C cycle.

GENERAL INTRODUCTION

There is a lot of scientific evidence showing that anthropogenic CO_2 emissions have caused the climate to change. According to Smith et al. (2009) on “Clearing the air”, the great Swedish chemist Svante Arrhenius, back in 1895, used basic physical concepts, already well understood at the time, to described how variations in trace gases on the atmosphere (particularly CO_2) should influence the heat budget on Earth. Therefore, a dramatic reduction on CO_2 emissions is essential to reduce the risk of future devastating effects. But even if we could halt anthropogenic C emissions today, the climate risk they pose would persist for a long time – longer than that of nuclear waste – assuming that we must rely on natural processes to dissipate the perturbation (Keith et al., 2009). As our modern society is highly dependent on energy, and around eighty percent of this energy is derived from fossil fuel (Chu, 2009), an immediate emissions halt is essentially impossible. Moreover, the development and implementation of cleaner sources of energy is something that takes time and money, what allow us to say that CO_2 emissions are going to keep high for a while.

This whole C issue has brought attention and a need to understand better the global C cycle. Soil is one of the largest compartments of C on its global cycle, with higher C content than plant biomass and atmosphere combined (Amundson, 2001). It is estimated that there are ~2344 gigatons of C storage as soil organic C (SOC) at the first three meters of soils globally (Jobággy and Jackson, 2000). Besides its size, SOC compartment is also very dynamic, with each C atom in atmospheric CO_2 passing through SOC somewhere in the world every ~12 years (Amundson, 2001). To make it happen, atmospheric CO_2 must be sequestered by terrestrial plants through photosynthesis. Then the plant needs to die and be incorporated to the soil, and lastly the plant material needs to be transformed by microorganisms into more stable forms known as humus. Thus, photosynthesis is a process of great importance to the global C cycle, with terrestrial vegetation sequestering a quarter of all CO_2 released annually into the atmosphere (Normile, 2009).

With those issues in mind, the goal of this work was to improve the knowledge about the global C cycle, studying SOC quantitative and qualitatively. In order to have a better understanding about the factors that lead to SOC stabilization, we focused at three different stages of it. On chapter 1 we did a meta-analysis on global resorption efficiencies and concentrations of carbon and nutrients in terrestrial plants. Nutrient resorption is a key component of plant nutrient conservation strategies and hence of productivity and elemental cycling in ecosystems. It influences many, if not most, ecosystem processes, including carbon cycling and resource-use efficiency (Aerts and Chapin 2000; Jackson et al. 2000; Gleason and Ares 2007; McGroddy et al. 2004). Thus, improved estimates of resorption parameters are needed for predicting long-term primary productivity and for improving biogeochemical models. Overall, global estimates for nutrient concentrations and resorption efficiencies should improve models that explicitly represent the cycling of C and nutrients, allowing the modeling community to represent more explicitly the coupling of other nutrient cycles.

On chapter 2 we performed the thermodynamic study of humic substances, which are found in nearly all soils, sediments, and natural waters. They make up roughly all the soil organic carbon and play a key role in many, if not most, chemical and physical properties in their environment. Besides of their importance, high complexity of humic substances makes them a poorly understood system. In order to understand the fundamental behavior and to obtain structural details of the humic substances we performed a thermodynamic study of them through isothermal titration calorimetry (microcalorimetry) technique using cationic surfactants as molecular probes. Comprehending humic substances is essential to improve soil organic carbon storage and the fate of other compounds when interacting with them.

On chapter 3 we analyzed SOC storage in three major Brazilian biomes under native vegetation, eucalyptus, and pasture, studying the association of SOC with climatic conditions and clay content. This type of study is justified by the fact that Brazilian C emissions' pattern is different from those of the developed countries. In Brazil, the majority of greenhouse gases emissions come from land use change and agriculture. Other than that, in order to improve overall estimates of SOC globally, regional studies are needed, especially in Brazil, where such data are still scarce (Bernoux et al., 2002). Also, the global role of SOC and its sensitivity to climatic conditions are still not well understood (Gianelle et al., 2010).

CHAPTER 1
Submitted as original paper to *Ecological Monographs*

GLOBAL RESORPTION EFFICIENCIES AND CONCENTRATIONS OF CARBON AND NUTRIENTS IN LEAVES OF TERRESTRIAL PLANTS

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ABSTRACT

Nutrient resorption in plants influences nutrient availability and cycling and is a key process in biogeochemical models. Improved estimates of resorption parameters are needed for predicting long-term primary productivity and for improving such models. Currently, most models assume a value of 50% resorption for nitrogen (N) and phosphorus (P) and lack resorption data for other nutrients and for different vegetation types. We provide global estimates of resorption efficiencies and nutrient concentrations for carbon (C), N, and P and the first global-scale estimates for essential nutrients such as potassium (K), calcium (Ca), and magnesium (Mg). We also examine mass loss during senescence, determining leaf mass loss (LML) globally and for each plant type, thus defining a mass loss correction factor (MLCF) needed to quantify *in situ* decomposition and resorption. We used a global meta-analysis of 86 studies and ~1,000 data points across climates for green and senesced leaves in six plant types: ferns, forbs, graminoids, conifers, and evergreen and deciduous woody angiosperms. In general, N and P resorption differed significantly from the commonly used global value of 50% (62.1 and 64.9%, respectively; $P<0.05$). Ca, C, and Mg showed lower average resorptions of 10.9, 23.2, and 28.6%, respectively. In contrast, K had the highest resorption of all nutrients studied (70.1%). We also found that resorption of N, P, and Ca depended on leaf nutrient-status; globally, N and P showed a decrease in resorption with increased nutrient status, while Ca showed an increase. On average, global leaf mass loss was 24.2%. Overall, our resorption data differ substantially from commonly assumed values and should help improve biogeochemical and land-surface models.

Keywords: Biogeochemical and land-surface models, Calcium, Carbon, Leaf mass loss, Magnesium, Nitrogen, Nutrient resorption efficiency, Phosphorus, Potassium.

1. INTRODUCTION

Nutrient resorption (NuR) is a key component of plant nutrient conservation strategies and hence of productivity and elemental cycling in ecosystems. It influences many, if not most, ecosystem processes, including carbon cycling and resource-use efficiency (Aerts and Chapin 2000, Jackson et al. 2000, Gleason and Ares 2007, McGroddy et al. 2004), plant litter decomposition through changes in litter quality (Berg and McClaugherty 2007, Manzoni et al. 2008, Manzoni et al. 2010), and plant competition (Eckstein et al. 1999, Yuan et al. 2005). From a physiological perspective, resorption from leaves is the net result of several processes, including the enzymatic breakdown of N- and P-containing compounds in leaves, phloem loading and transport, and the formation of an abscission layer that severs the transport (Lambers et al. 1998). NuR is also an energetically expensive process influenced by physiological factors and nutrient economics (Gordon and Jackson 2000, Freschet et al. 2010). From the perspective of leaf economics, understanding how NuR works and providing good quantitative estimates of its occurrence are essential for modeling nutrient cycling, biosphere productivity, and responses of plants and the biosphere to a changing climate (Jackson et al. 1997, Wright et al. 2004). In particular, the new generation of coupled global models of the carbon cycle and climate system require reliable estimates of resorption efficiencies globally (Thornton et al. 2007).

Although some studies have examined plant NuR in relation to climate, soil characteristics, and plant traits, data limitations have made the search for mechanistic and global relationships difficult (Chapin and Moilanen 1991, Aerts 1996, Lambers et al. 1998, Aerts and Chapin 2000, Kazakou et al. 2007, Yuan and Chen 2009a). N resorption (NR) generally increases from the tropics to the tundra while P resorption (PR) decreases, mirroring increased N- and decreased P-limitation towards northern latitudes (Yuan and Chen 2009a). High NuR was predicted to be more common in low-fertility soils, but this relationship has not been universally supported (Aerts 1996, Diehl et al. 2003). Similarly, an expected correlation between soil moisture and nutrient retention was not observed when tested in a tree species, *Austrocedrus chilensis* (Buamscha et al. 1998). Much less is known about resorption patterns of other essential nutrients, particularly K, Mg, and Ca, which to our knowledge have not been studied globally.

Results for the relationship of resorption efficiency and plant nutrient status have also been contradictory. While some studies did not find any relationship (Chapin and Moilanen 1991, Reich et al. 1992, Aerts 1996, Lambers et al. 1998, Aerts and Chapin 2000, Kazakou et al. 2007, Yuan and Chen 2009a), others have found resorption efficiency to be related to plant nutrient status (Aerts 1996, Lal et al. 2001, Wright and Westoby

2003, Kobe et al. 2005, Cai and Bongers 2007). Although resorption efficiency sometimes is positively correlated with both N concentrations in mature leaves and soil N fertility (Diehl et al. 2003), a study by van Heerwaarden *et al.* (2003) found a general trend towards decreased NuR efficiency with higher N supply. Nutrient conservation strategies have also been related to plant functional type (Diehl et al. 2003, Yuan and Chen 2009a), although observed differences across growth forms and functional groups have typically been small. Where differences have been observed, NR tends to be higher in deciduous than in evergreen species and in trees than in shrubs (Yuan and Chen 2009b). PR is generally higher in graminoids (Aerts 1996) and in evergreen than deciduous species (Yuan and Chen 2009b).

Along with its ecological importance in the field, nutrient resorption parameters are also vital for the accuracy of ecosystem and biogeochemical models. Our analysis of 25 such models shows wide variation in NuR values, from 0 to 90% of N resorption, with the most commonly used estimate of resorption efficiency being 50% (Table 1). This model overview also highlights some limitations in current resorption parameterization. First, ecosystem models tend to neglect the dynamics of P and do not consider other nutrients. Second, few of these models include different resorption parameters for different plant functional types, even when they model those functional types explicitly. Thus, a more solid observational base of NuR efficiencies could help constrain nutrient cycling in coupled biogeochemical models.

Published NuR estimates are strongly affected by differences in measurement approaches. Most works express nutrient pools on a leaf-mass basis. An inherent problem is that mass loss occurs during senescence, changing the measurement basis and leading to underestimates of NuR (van Heerwaarden et al. 2003). Several approaches have been used to avoid this issue. Nutrient pools have been expressed on the basis of leaf area or length, Ca concentration, lignin content, canopy area, and more, assuming that these factors do not change during senescence. However, changes do occur in most cases (e.g., leaf shrinkage) and the only unbiased method to estimate resorption is based on measurement of nutrient pools in the same leaves before and after senescence. Because this approach is impractical, other methods are employed, recognizing the need to quantify any corresponding errors. For example, leaf mass loss could lead to an average NuR underestimation of 10% when using leaf mass-based concentrations, while leaf shrinkage could lead to an average underestimation of 6% when using area-based concentrations (van Heerwaarden et al. 2003). For these reasons, global-scale correction factors are needed that account for changes in leaf mass (or area) during senescence and that can be used to obtain unbiased estimates of resorption.

The goal of our work was to identify fundamental trends in NuR and element concentrations for different plant functional groups and climatic variables. We also examined leaf mass loss during senescence, which influences estimated NuR. We assembled a database of 996 data points from 86 studies to perform a meta-analysis of nutrient contents in mature and senesced leaves, and resorption from unfertilized vegetation. Our objectives were to: (1) improve existing estimates of resorption efficiencies for C, N, and P; (2) provide the first global estimates of resorption efficiencies for K, Ca and Mg; (3) analyze resorption efficiencies of C, N, P, K, Ca and Mg for different climatic regions, soil fertility, and plant functional groups, (4) determine if N-fixers had lower N and higher P resorption, (5) characterize nutrient contents in leaves globally, and (6) characterize leaf mass loss during senescence globally.

Table 1. Leaf N and P resorption efficiencies (NR and PR) as represented in ecosystem and global biogeochemical models.

Model name	Source	NR[1]	PR[1]	Notes
PHOENIX	(McGill et al. 1981)	0.8	-	
JABOWA	(Pastor and Post 1986)	X	-	
Hurley	(Thornley and Verberne 1989)	~0.5-0.9	-	Resorption depends on plant N concentration
GEM	(Hunt et al. 1991)	~0-0.5	-	Resorption depends on plant N concentration (lower leaf N, lower resorption)
VEGIE	(Aber et al. 1991)	0	-	
MBL-GEM	(Rastetter et al. 1991)		-	Resorption is constant but value is not reported
FOREST-BGC	(Running and Gower 1991)	0.5	-	Generic value for all ecosystems
TEM	(Raich et al. 1991)	X	-	No distinction among leaves, stem, and roots
-	(Rastetter and Shaver 1992)	Variable	-	Resorption depends on plant N concentration (lower leaf N, lower resorption)
CASA	(Potter et al. 1993)	X	-	Litter C:N depends on plant functional type
G'DAY	(Comins and McMurtrie 1993)	0	-	
CENTURY	(Parton et al. 1993)	X	X	
-	(Aerts and van der Peijl 1993)	0.1-0.18	-	Species-specific values
NICCCE	(van Dam and van Breemen 1995)	0.2-0.3	-	Resorption may vary as a function of leaf N
-	(Schwinning and Parsons 1996)	0	-	
TREEDYN3	(Bossel 1996)	0.15-0.4	-	Species-specific values
-	(Tateno and Chapin 1997)	0.7	-	
HYBRID III	(Friend et al. 1997)	0.5	-	
IBIS-II	(Kucharik et al. 2000)	X	-	Litter C/N is constant across functional types
BIOME-BGC	(White et al. 2000)	0.45-0.67	-	Biome-specific values[2]; dead wood has different C:N from live wood
-	(Daufresne and Loreau 2001)	0	-	Estimated as ratio of total plant C to total plant N turnover rates
-	(Baisden and Amundson 2003)	0	-	
LPJ	(Sitch et al. 2003)	0	-	
-	(Wang et al. 2007)	0.5	0.5	Only ecosystem model with P dynamics
ISAM	(Yang et al. 2009)	0.5	-	Generic value for all ecosystems

[1] X indicates that resorption efficiency varies because litter C:N is constant and plant C:N varies depending on the relative uptake of N or C by the plant. [2] NR=0.55 for deciduous broadleaf and evergreen needle forests, 0.67 for deciduous needle forest, 0.45 for grasses, and 0.53 for shrubs.

2. MATERIALS AND METHODS

2.1 Data description

We conducted a global meta-analysis of published studies for C, N, P, K, Ca, and Mg concentrations and dry mass of green and senesced leaves to estimate NuR efficiencies and leaf mass loss of terrestrial plants during senescence. We compiled data on nutrient contents in green and senesced leaves from 86 studies in 31 countries on every continent except Antarctica, with the most data points from Europe and North America and the fewest from Russia and Africa (Figure 1). We collected data from studies that reported or allowed us to calculate mean values of nutrient mass per unit dry mass in mature green and senesced leaves and report nutrient content on a leaf-mass basis. Most of the data for senesced leaves came from newly fallen leaves, while a small subset of data were collected from litter-trap studies. Although nutrient leaching may occasionally lead to underestimates of nutrient concentrations in senesced leaf litter (Yuan et al. 2005), an intensive leaching experiment for 40 species found that leaching accounted for no more than one one-hundredth of the leaf N pool, and less than one ten-thousandth of the leaf P pool, with the average N and P resorption, respectively, 55-fold and $>10^4$-fold higher than potential N and P leaching losses (Freschet et al. 2010).

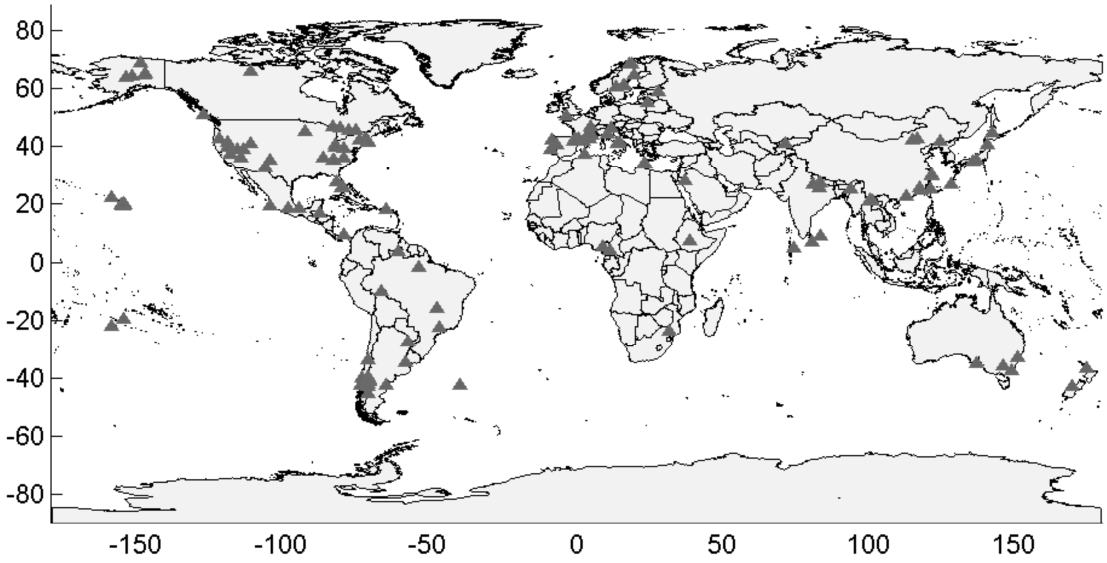

Figure 1. Global distribution of the nutrient resorption data set.

We obtained data from major terrestrial vegetation types, including ferns, forbs, graminoids, and woody species (lianas, shrubs, and trees), grouped in six growth types: ferns, forbs, graminoids, conifers, evergreen woody angiosperms, and deciduous woody angiosperms. We also determined which species were N-fixers, to compare concentration and resorption properties to those of non-N-fixing species. Data for fertilized systems, including annual crops, were excluded from the analysis. For any studies on fertilized systems included here, we used only the unfertilized controls. Wherever possible, we obtained mean annual temperature (MAT) and mean annual precipitation (MAP) data and field characteristics for each site. Across the global dataset, site MAT ranged from -8.0 to 31.6 ^{o}C, MAP ranged from 125 to 5500 mm per year, and altitude ranged from 0 to 3520 m above sea level. Based on these climatic features we grouped our data according to Köppen’s climate classification: A – Tropical/megathermal; B – Dry (arid and semiarid, including desert and steppe climates, where precipitation is less than the potential evapotranspiration); C – Temperate/mesothermal (including Mediterranean, Oceanic, Humid Subtropical and Sub-polar Oceanic climates); D – Continental/microthermal and; E – Polar. This classification links native vegetation to climate by combining average annual and monthly temperatures and precipitation, as well as the seasonality of precipitation (McKnight and Hess 2000), and it is still the most frequently used climate classification (Kottek et al. 2006). Overall, 171 data points were available for C concentrations in leaves, 948 for N, 669 for P, 207 for K, 150 for Ca, 115 for Mg, and 191 for leaf mass-loss calculations (APPENDIX). Relatively few of the studies provided data on soil attributes, but where possible we compiled data for extractable soil nutrients and texture.

2.2 *Resorption calculation and data analysis*

Resorption data are often presented as NuR efficiency, defined as the proportional withdrawal of a nutrient during senescence (Cartaxana and Catarino 2002, van Heerwaarden et al. 2003, Wright and Westoby 2003, Cai and Bongers 2007, Yuan and Chen 2009a),

$$\mathrm{NuR} = \left(1 - \frac{\text{Mass of nutrient in senesced leaves}}{\text{Mass of nutrient in green leaves}}\right) \cdot 100 \qquad (1)$$

Most observations, however, are made on a leaf mass basis, and resorption is thus estimated as

$$\mathrm{NuR} = \left(1 - \frac{\mathrm{Nu}_{\mathrm{Sen}}}{\mathrm{Nu}_{\mathrm{Gr}}}\right) \cdot 100 \qquad (2)$$

where

$$\mathrm{Nu}_{\mathrm{Gr}} = \frac{\text{Mass of nutrient in green leaves}}{\text{Dry mass of green leaves}} \qquad (3)$$

$$\mathrm{Nu}_{\mathrm{Sen}} = \frac{\text{Mass of nutrient in senesced leaves}}{\text{Dry mass of senesced leaves}} \qquad (4)$$

Clearly, using this method leads to unbiased estimates of the real resorption (Eq. 1) only if leaf mass does not change during senescence. However, leaf mass loss (LML) can be as high as 40% (Kazakou et al. 2007), leading to an underestimation of nutrient resorption because of increased Nu_{Sen}, especially when comparing species that lose different relative amounts (van Heerwaarden et al. 2003). To obtain unbiased resorption estimates, we can rewrite the real resorption as

$$\mathrm{NuR} = \left(1 - \frac{\mathrm{Nu_{Sen}}}{\mathrm{Nu_{Gr}}}\mathrm{MLCF}\right) \cdot 100 \tag{5}$$

where MLCF is the mass loss correction factor, specifically the ratio of the dry mass of green leaves, M_{Gr}, and the dry mass of senesced leaves, M_{Sen},

$$\mathrm{MLCF} = \frac{\mathrm{M_{Sen}}}{\mathrm{M_{Gr}}} \tag{6}$$

Using MLCF, we corrected nutrient contents in senesced leaves by normalizing them by green leaf mass,

$$\mathrm{Nu^{*}_{Sen}} = \mathrm{Nu_{Sen}}.\mathrm{MLCF} \tag{7}$$

Accordingly, the unbiased estimate of resorption efficiency (analytically equivalent to the definition of NuR in Eq. (1)) is given by:

$$\mathrm{NuR} = \left(1 - \frac{\mathrm{Nu^{*}_{Sen}}}{\mathrm{Nu_{Gr}}}\right).100 \tag{8}$$

The MLCF was calculated directly when data on dry mass were shown for both green and senesced leaves. When just the percentage of leaf mass loss (LML) was shown, we calculate MLCF as

$$\mathrm{MLCF} = 1 - \frac{\mathrm{LML}}{100} \tag{9}$$

Based on the obtained MLCF, we used Eq. (8) to estimate the NuR for each data point and species, comparing the values with other leaf traits. MLCF were estimated for each growth type studied here, except for ferns, because we could not find any data on LML for them. Therefore, the corrections for the unbiased resorption efficiency were done for each growth type separately. This approach means that each growth type had its own MLCF. For ferns, MLCF was estimated from the whole LML dataset.

To calculate mean nutrient resorption ($\overline{\mathrm{NuR}}$) for different functional groups or the global dataset as a whole, and to assess the role of plant nutrient status on resorption efficiency, we used power law regressions according to Kobe et al. (2005):

$$\mathrm{Nu}^{*}_{\mathrm{Sen}} = \alpha \mathrm{Nu}^{\beta}_{\mathrm{Gr}} \tag{10}$$

which corresponds to a linear regression in the logarithmic plot,

$$\log\left(Nu^{*}_{Sen}\right) = \log(\alpha) + \beta \log\left(Nu_{Gr}\right) \tag{11}$$

Combining Eqs (8) and (10) yields the following expression for $\overline{\mathrm{NuR}}$,

$$\overline{\mathrm{NuR}} = \left(1 - \alpha\, \mathrm{Nu}^{\beta-1}_{\mathrm{Gr}}\right).100 \tag{12}$$

For Eq. 12, a β value >1 indicates the cases where nutrient resorption efficiency decreases with leaf nutrient status. In other words, when $\beta>1$, resorption is more efficient in green leaves that have low nutrient concentrations. In contrast, $\beta<1$ indicates higher resorption efficiency in nutrient-rich fresh leaves. Eq. (10) was used first to assess the mean nutrient resorption ($\overline{\mathrm{NuR}}$) independently of nutrient status, with the β exponent was set to 1, resulting in a linear correlation. Second, we assessed the role of leaf nutrient status by determining β through nonlinear regression. This two-step regression allowed us to compare our results to the $\overline{\mathrm{NuR}}$ values based on linear regression that are typically reported, while also considering, as a second-order approximation, the effect of nutrient status.

The data were log-transformed for statistical analyses to correct heteroscedasticity in the dataset. We used the reduced major axis (RMA) regression analysis (type II regression) with a logarithmic transformation, a common approach in allometric and stoichiometric studies (Seim and Sæther 1983, Kobe et al. 2005, Niklas 2006). According to Niklas (2006), when a predictive relationship is sought, simple ordinary least squares (OLS) regression analysis (also known as type I regression) can be used. However, when the objective is to establish a functional relationship between x

and *y*, as is generally the case, RMA should be used. OLS regression is based on the assumption that *x*-values are known exactly, while only the *y*-values (dependent variable) are subject to measurement error (Seim and Sæther 1983). In biological datasets, in contrast, *x* and *y*-values are often subject to measurement errors of comparable magnitude. Graphically, RMA minimizes perpendicular offsets, incorporating uncertainties of the data points along both *x* and *y*-axes. For purposes of comparison, we proceed with both regression types (I and II) analysis, but results from type II regression are shown here.

We calculated $\overline{\text{NuR}}$ for each nutrient for the dataset as a whole and for each plant type and climate group separately. To compare $\overline{\text{NuR}}$ for the entire dataset and for each vegetation type and climate group, we used 95% confidence intervals of the regressions. Differences in growth types as well as differences with 0 or 50% resorption cited throughout the text were assessed as non-overlapping 95% confidence intervals. Similarly, the average amounts of nutrients in green and senesced leaves are presented as mean content followed by 95% confidence intervals. To estimate relationships between NuR and climate variables (MAT and MAP) and latitude, we used Pearson correlations.

3. RESULTS

3.1 Leaf mass loss (LML) and mass-loss correction factors (MLCF)

On average, leaves lost 24.2% of their mass during senescence (Table 2). Within growth types, LML ranged from 21.6% (deciduous woody angiosperms) to 36.0% (forbs). There was a strong correlation between mass in green and senesced leaves for all plant functional types (Figure 2). The global β value was slightly, but statistically, >1 (β =1.04), meaning that lighter leaves lose slightly more mass proportionally than heavier leaves did. Based on the LML data, we calculated MLCF

(Eq. 9) and used it to correct nutrient resorption estimates for mass loss during senescence (Eqs. 8 and 12).

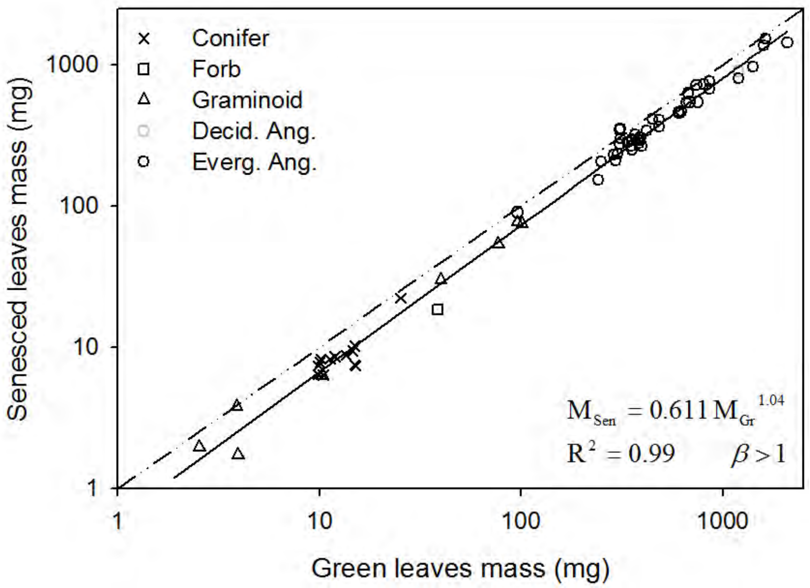

Figure 2. Leaf mass loss (LML) during senescence. Green leaves mass *vs* senesced leaves mass on a log scale.

Table 2. Leaf mass loss (LML), mass loss correction factor (MLCF), and average C and nutrients content (% dry mass) in green and senesced leaves (not corrected for mass loss), followed by 95% confidence interval, for the entire data set and for different plant growth types.

	All data	Ferns	Forbs	Gram.	Conif.	Everg. ang.	Dec. ang.
LML	24.2 ±2.1	-	36.0 ±7.5	28.7 ±5.5	25.5 ±6.8	22.0 ±2.9	21.6 ±3.9
MLCF	0.762	-	0.640	0.713	0.745	0.780	0.784
n	191	-	18	18	24	68	63
C_{GR}	44.0 ±0.68	-	-	44.4 ±2.09	49.8 ±8.96	44.62 ±1.00	42.99 ±0.99
C_{SEN}	43.3 ±0.80	-	-	41.4 ±1.97	54.2 ±10.02	44.61 ±1.19	41.77 ±1.05
n	171	-	-	15	4	78	74
N_{GR}	1.840 ±0.050	1.335 ±0.276	2.115 ±0.258	1.941 ±0.167	1.138 ±0.087	1.725 ±0.079	2.033 ±0.071
N_{SEN}	0.974 ±0.033	0.808 ±0.198	1.092 ±0.164	0.739 ±0.084	0.590 ±0.057	1.000 ±0.054	1.071 ±0.051
n	948	22	88	83	81	307	367
P_{GR}	0.143 ±0.007	0.136 ±0.030	0.158 ±0.033	0.191 ±0.043	0.096 ±0.013	0.125 ±0.011	0.155 ±0.011
P_{SEN}	0.077 ±0.006	0.065 ±0.017	0.078 ±0.022	0.060 ±0.018	0.045 ±0.014	0.073 ±0.009	0.092 ±0.009
n	669	22	58	51	53	222	263
K_{GR}	0.955 ±0.087	-	1.701 ±0.468	1.265 ±0.323	0.418 ±0.092	0.879 ±0.117	0.924 ±0.092
K_{SEN}	0.471 ±0.067	-	0.998 ±0.400	0.281 ±0.091	0.132 ±0.044	0.576 ±0.139	0.417 ±0.053
n	207	-	24	15	30	49	89
Ca_{GR}	1.110 ±0.124	-	1.856 ±0.382	0.333 ±0.102	0.380 ±0.054	1.157 ±0.221	1.202 ±0.166
Ca_{SEN}	1.318 ±0.144	-	1.907 ±0.503	0.311 ±0.105	0.518 ±0.128	1.491 ±0.241	1.462 ±0.198
n	150	-	22	16	15	43	54
Mg_{GR}	0.336 ±0.054	-	0.506 ±0.116	0.099 ±0.015	0.078 ±0.011	0.367 ±0.087	0.398 ±0.130
Mg_{SEN}	0.348 ±0.059	-	0.524 ±0.121	0.084 ±0.013	0.061 ±0.009	0.433 ±0.103	0.365 ±0.134
n	115	-	22	12	15	36	30

n = number of observations; Nu_{GR} = nutrient content (%) in green leaves; Nu_{SEN} = nutrient content (%) in senesced leaves (not corrected for mass loss); LML = leaf mass loss during senescence (%); and MLCF = mass loss correction factor (i.e., MLCF = (senesced leaf mass/green leaf mass)).

Physical leaf properties in both green and senesced leaves (e.g., specific leaf area (SLA), leaf area, and leaf mass) showed significant correlations with latitude, mean annual temperature (MAT), and mean annual precipitation (MAP), but showed no relationship with LML (see APPENDIX). SLA, leaf area and leaf mass showed negative correlations with latitude (*P*<0.001) but strong, positive correlations with MAT (*P*<0.01). Only leaf mass showed positive correlation with MAP (*P*<0.01).

3.2 *Nutrient content and mean resorption efficiency (* $\overline{\mathrm{NuR}}$ *) across plant functional types*

Across the global dataset of C, N, P, K, Ca, and Mg concentrations and plant growth forms, forbs always had the highest nutrient concentrations or among the highest for both green and senesced leaves (Table 2). In contrast, conifers generally had the lowest nutrient contents in both green and senesced leaves (Table 2). For $\overline{\mathrm{NuR}}$, graminoids were always among the growth types with the highest $\overline{\mathrm{NuR}}$ while evergreen woody angiosperms typically had the lowest or close to lowest $\overline{\mathrm{NuR}}$.

Mean N and P resorptions ($\overline{\mathrm{NR}}, \overline{\mathrm{PR}}$) globally were 62.1% and 64.9%, respectively. These values are statistically greater than the typically assumed value of 50% (*P*<0.05; Figure 3). Within plant types, graminoids and forbs had the highest $\overline{\mathrm{NR}}$ (74.6 and 70.2%, respectively) while evergreen woody angiosperms and ferns had the lowest values (56.1 and 59.2%, respectively) (Figure 4). For $\overline{\mathrm{PR}}$, evergreen and deciduous woody angiosperms had the lowest resorption (58.4 and 58.5%, respectively), whereas conifers and graminoids showed the highest $\overline{\mathrm{PR}}$ (77.0 and 82.1%, respectively).

Average nutrient resorption for K ($\overline{\mathrm{KR}}$) of 70.1% was the highest for all nutrients examined (Figure 3). As was the case for N and P, graminoids, together with conifers, showed the highest $\overline{\mathrm{KR}}$ values (84.9 and 79.1%, respectively) (Figure 4), while evergreen woody angiosperms and forbs had the lowest $\overline{\mathrm{KR}}$ (56.1 and 71.1%, respectively).

In general, C, Ca, and Mg showed a lower average resorption (23.2, 10.9, and 28.6%, respectively) compared with the other nutrients (Figure 3), and none of the three showed any evidence of enrichment during senescence. $\overline{\mathrm{MgR}}$ was found to be the most conservative and was statistically indistinguishable among all growth types except for evergreen woody angiosperms, which had the lowest value (*P*<0.05; 11.7%). $\overline{\mathrm{CaR}}$ was statistically indistinguishable from 0% resorption for all woody species, but graminoids and forbs had $\overline{\mathrm{CaR}}$ of 32.5 and 36.9%, respectively (*P*<0.05; Figure 4). For C, graminoids showed the highest mean resorption (33.6%) whereas conifers and evergreen woody angiosperms had the lowest values, 18.9 and 20.8%, respectively (Figure 4).

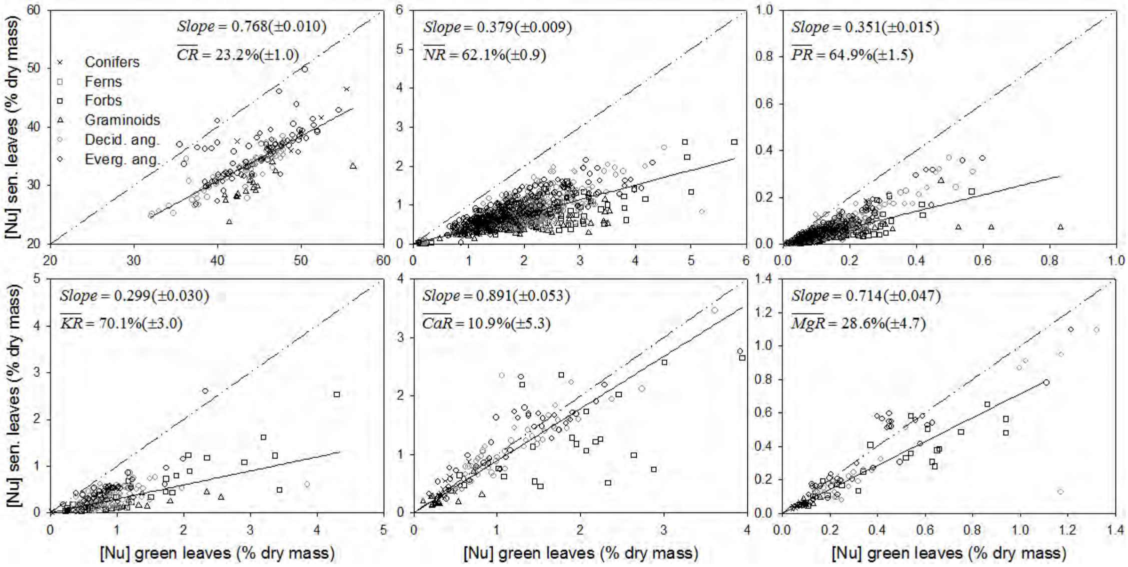

Figure 3. Linear regressions of nutrient content in green *vs* senesced leaves (corrected for the mass loss) for the global data set. Dash-dotted line is the reference line set to a unitary slope, defining zero resorption.

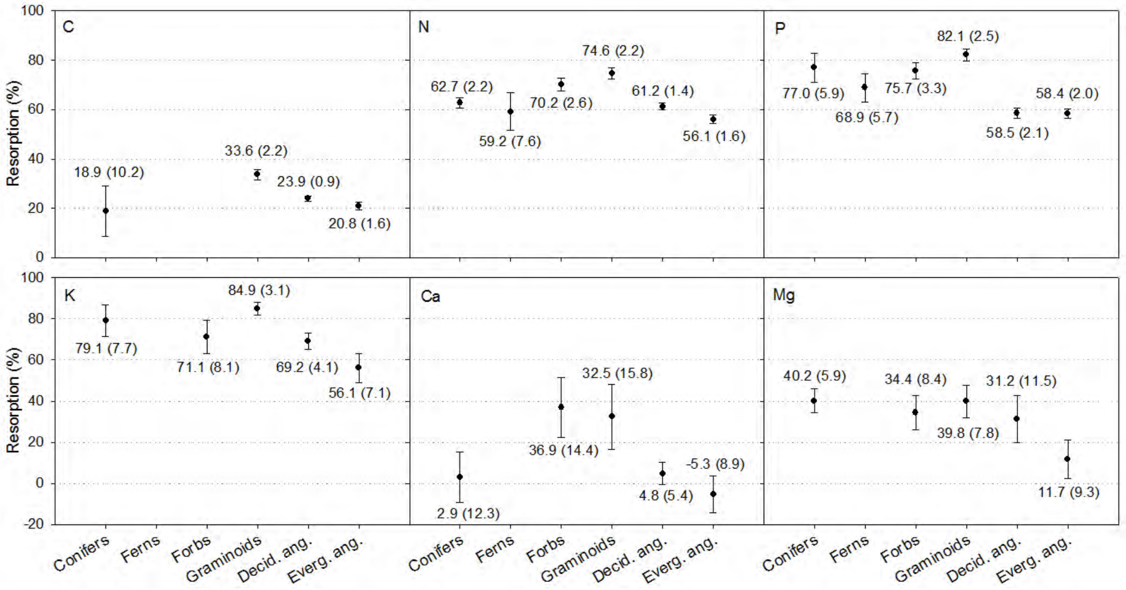

Figure 4. Mean nutrient resorption by growth type for all nutrients studied. Values under or above symbols are the mean nutrient resorption for each given point. Within parentheses are the 95% confidence intervals.

3.3 Nutrient content and mean nutrient resorption for N_2 fixers and non-fixers.

In general, N_2 fixers have been shown to have higher N concentrations in both green and senesced leaves compared to non-fixers and to have lower N resorption (Killingbeck 1996, Killingbeck and Whitford 2001, Wright and Westoby 2003). In agreement with such studies, we found that the content of N in both green and senesced leaves was higher in N_2 fixers. In contrast, we did not find statistically significant differences in the contents of C and other nutrients in green and senesced leaves between N_2 fixers and non-fixers plants (Table 3).

Table 3. Average C and nutrients content (% dry mass) in green and senesced leaves (not corrected for mass loss) for N_2 fixers and non-fixers species, followed by its 95% confidence interval.

	Fixers				Non-fixers			
	All	Forbs	Decid. ang.	Everg. ang.	All	Forbs	Decid. ang.	Everg. ang.
C_{GR}	44.89 ±1.83	-	44.23 ±1.75	-	43.75 ±0.75	-	42.84 ±1.1	44.54 ±1.02
C_{SEN}	43.15 ±1.86	-	42.63 ±1.99	-	43.26 ±0.87	-	41.67 ±1.16	44.59 ±1.22
N_{GR}	2.641 ±0.202	3.035 ±0.547	2.365 ±0.245	2.314 ±0.469	1.872 ±0.057	2.023 ±0.272	1.995 ±0.074	1.695 ±0.077
N_{SEN}	1.552 ±0.145	1.552 ±0.418	1.522 ±0.209	1.624 ±0.243	1.001 ±0.037	1.046 ±0.175	1.019 ±0.050	0.969 ±0.053
P_{GR}	0.142 ±0.026	-	0.132 ±0.029	0.145 ±0.063	0.143 ±0.008	0.153 ±0.034	0.158 ±0.012	0.123 ±0.012
P_{SEN}	0.074 ±0.018	-	0.072 ±0.025	0.078 ±0.035	0.084 ±0.006	0.078 ±0.023	0.095 ±0.009	0.073 ±0.009
K_{GR}	0.813 ±0.207	-	0.671 ±0.111	1.127 ±0.696	1.048 ±0.108	1.701 ±0.468	0.959 ±0.102	0.851 ±0.117
K_{SEN}	0.511 ±0.152	-	0.396 ±0.146	0.764 ±0.330	0.556 ±0.088	0.998 ±0.400	0.419 ±0.059	0.555 ±0.153
Ca_{GR}	1.310 ±0.290	-	-	-	1.306 ±0.150	1.856 ±0.383	1.169 ±0.188	1.150 ±0.245
Ca_{SEN}	1.768 ±0.464	-	-	-	1.526 ±0.165	1.907 ±0.503	1.382 ±0.208	1.476 ±0.258
Mg_{GR}	0.518 ±0.319	-	-	-	0.398 ±0.059	0.506 ±0.117	0.357 ±0.121	0.352 ±0.081
Mg_{SEN}	0.543 ±0.343	-	-	-	0.417 ±0.064	0.524 ±0.121	0.309 ±0.118	0.424 ±0.099

Similarly, $\overline{\text{NuR}}$ values were not different between fixers and non-fixers species except again for $\overline{\text{NR}}$, which was significantly lower in N_2 fixers ($P<0.05$; Table 4). Moreover, β values were always lower in N_2 fixers compared to non-fixers ($P<0.05$; Table 4). As for P, $\overline{\text{PR}}$ showed no difference between N_2 fixers and non-fixers, but for non-fixers $\overline{\text{PR}}$ depended upon P status ($\beta>1$, $P<0.05$), while this did not hold for N_2 fixers ($\beta=1$).

Table 4. Mean C and nutrients resorption (corrected for mass loss; followed by its 95% confidence interval) and β coefficients for N_2 fixers and non-fixers species.

	Fixers				Non-fixers			
	All	Forbs	Decid. ang.	Everg. ang.	All	Forbs	Decid. ang.	Everg. ang.
$\overline{CR}$	24.5 ±1.4	-	24.5 ±1.8	-	22.1 ±1.0	-	23.8 ±1.0	20.7 ±1.6
β	1.113	-	1.188	-	*1.165*	-	1.079	1.160
n	10	-	8	-	142	-	66	76
$\overline{NR}$	52.2 ±4.0	68.4 ±7.3	51.6 ±4.9	43.0 ±5.9	61.0 ±1.1	70.4 ±2.8	62.1 ±1.5	56.6 ±1.7
β	1.140	1.714	1.200	0.985	*1.289*	*1.229*	*1.301*	*1.299*
n	61	8	37	15	701	80	329	292
$\overline{PR}$	64.1 ±5.2	-	63.2 ±6.5	62.2 ±12.6	60.5 ±1.5	75.4 ±3.5	57.8 ±2.2	58.1 ±2.1
β	*1.334*	-	*1.315*	1.427	*1.306*	*1.253*	*1.277*	*1.291*
n	47	-	30	14	496	55	233	208
$\overline{KR}$	55.5 ±15.2	-	60.2 ±19.8	43.1 ±22.4	67.0 ±3.4	71.1 ±8.1	70.3 ±4.1	57.4 ±7.6
β	1.770	-	2.252	1.866	*1.612*	*1.419*	*1.795*	*1.832*
n	16	-	11	5	146	24	78	44
$\overline{CaR}$	-3.1 ±19.8	-	-	-	9.9 ±6.2	36.9 ±14.4	6.3 ±4.8	5.6 ±9.4
β	1.179	-	-	-	0.915	1.075	0.954	0.943
n	14	-	-	-	105	22	45	38
$\overline{MgR}$	15.4 ±11.0	-	-	-	25.9 ±6.7	34.4 ±8.4	34.9 ±13.3	10.9 ±10.6
β	0.878	-	-	-	1.104	0.954	0.980	1.253
n	11	-	-	-	77	22	24	31

β coefficients in italic are significant different from 1 ($P<0.05$), and n = number of data points.

3.4 Resorption efficiency and plant nutrient status

Based on the power law regression equation (Eq. 10), we tested whether nutrient resorption was affected by nutrient concentrations in green leaves ($\beta \neq 1$; Figure 5), even if not always apparent in Figure 3. For the entire dataset, almost all nutrients showed $\beta > 1$ (i.e., resorption efficiency decreased with nutrient status of the fresh leaves; $P<0.05$). Ca was the only element for which β did not differ statistically from one, suggesting no evidence for a relationship with nutrient status (Figure 5).

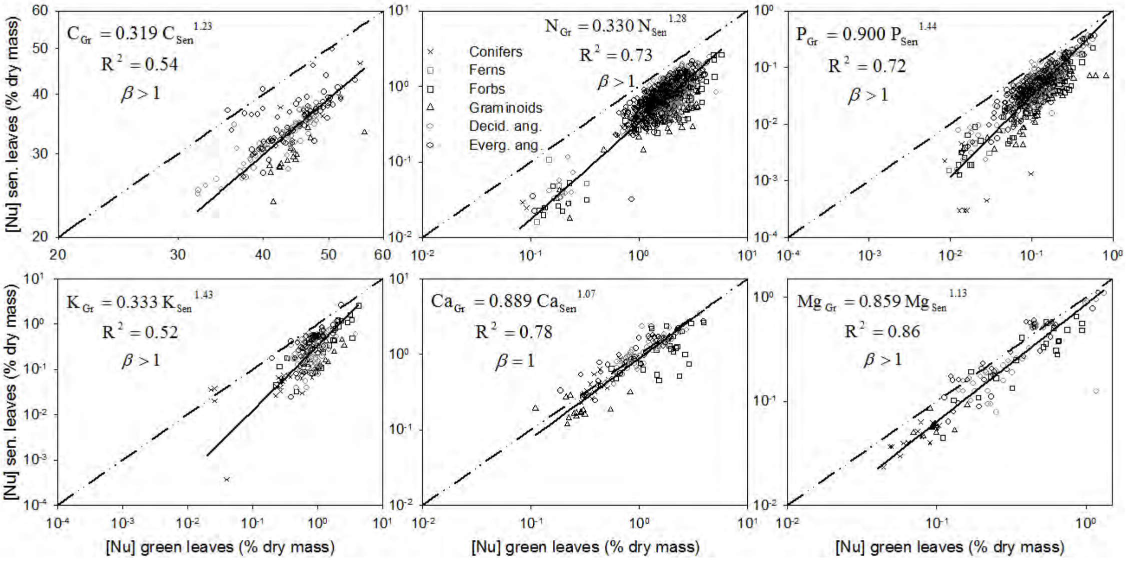

Figure 5. Power law regression analysis (Eq. 10) of C and nutrients content in green *vs* senesced leaves (corrected for mass loss) for the global data set on a log scale. β different from 1 means that the relationship between Nu_{Sen} and Nu_{Green} is not linear, with $\beta>1$ indicating decreased resorption efficiency with increasing leaf nutrient status.

When data were grouped by plant type or climate, however, the effect of nutrient status differed by variable. N and P resorption had $\beta>1$ for all growth types and climates ($P<0.05$; Figure 5; Tables 5 and 6). P resorption showed the highest β values in the dataset, 2.08 and 2.54 for conifers and Koppen B (dry climates), respectively. In contrast, C resorption had an overall $\beta>1$ for the entire dataset, but the relationship was driven primarily by results in Koppen C climates (Figure 5 and Table 6). For K, Ca, and Mg, responses in β were intermediate and depended on the specific climate and vegetation types (Tables 5 and 6).

Table 5. β coefficients for the entire data set and for different growth types. These β coefficients are obtained from the power law regression $\mathrm{Nu}_{\mathrm{Sen}}^{*} = \alpha \mathrm{Nu}_{\mathrm{Gr}}^{\beta}$ (Eq. 10). β different from 1 means that the relationship between $\mathrm{Nu}_{\mathrm{Sen}}$ and $\mathrm{Nu}_{\mathrm{Green}}$ is not linear, with $\beta > 1$ indicating decreased resorption efficiency with increasing leaf nutrient status.

	Ferns	Forbs	Graminoids	Conifers	Everg. ang.	Decid. ang.
$\overline{\mathrm{CR}}$	-	-	1.082	1.056	1.153	1.078
$\overline{\mathrm{NR}}$	*1.288*	*1.228*	*1.239*	*1.177*	*1.311*	*1.297*
$\overline{\mathrm{PR}}$	*1.263*	*1.240*	*1.227*	*2.075*	*1.335*	*1.300*
$\overline{\mathrm{KR}}$	-	*1.422*	1.195	1.180	*1.770*	*1.829*
$\overline{\mathrm{CaR}}$	-	1.098	0.872	*1.652*	1.024	0.950
$\overline{\mathrm{MgR}}$	-	1.000	1.013	0.994	1.074	*1.185*

Values in italic are significantly different from 1 ($P<0.05$).

Table 6. β coefficients for different climate groups. These β coefficients are obtained from the power law regression of Eq. (10) ($\mathrm{Nu}_{\mathrm{Sen}}^{*} = \alpha \mathrm{Nu}_{\mathrm{Gr}}^{\beta}$). β different from 1 means that the relationship between $\mathrm{Nu}_{\mathrm{Sen}}$ and $\mathrm{Nu}_{\mathrm{Green}}$ is not linear, with $\beta>1$ indicating decreased resorption efficiency with increasing leaf nutrient status.

	Koppen A	Koppen B	Koppen C	Koppen D	Koppen E
$\overline{\mathrm{CR}}$	1.110	1.365	*1.163*	-	-
$\overline{\mathrm{NR}}$	*1.233*	*1.392*	*1.303*	*1.223*	*1.237*
$\overline{\mathrm{PR}}$	*1.445*	*2.539*	*1.619*	*1.371*	*1.495*
$\overline{\mathrm{KR}}$	1.114	0.660	*1.241*	*1.542*	1.107
$\overline{\mathrm{CaR}}$	0.878	0.997	*1.245*	*1.455*	0.863
$\overline{\mathrm{MgR}}$	0.992	0.705	1.281	*0.748*	0.944

Values in italic are significantly different from 1 ($P<0.05$). “ns” means non-significant.

3.5 Resorption efficiency along climatic gradients

N, P, and K resorption efficiencies ($\overline{\mathrm{NR}}$, $\overline{\mathrm{PR}}$, and $\overline{\mathrm{KR}}$) increased with latitude because of significant negative correlations with both mean annual temperature and precipitation ($P<0.01$; Figure 6; see APPENDIX). $\overline{\mathrm{CR}}$ was negatively correlated only with precipitation (*P*<0.001) but not with temperature. The opposite was true for $\overline{\text{MgR}}$, which showed a negative relationship with temperature (*P*<0.001), but no correlation with precipitation. $\overline{\text{CaR}}$ was the only variable to show a small but positive correlation with temperature (*P*<0.05) and no correlation with precipitation.

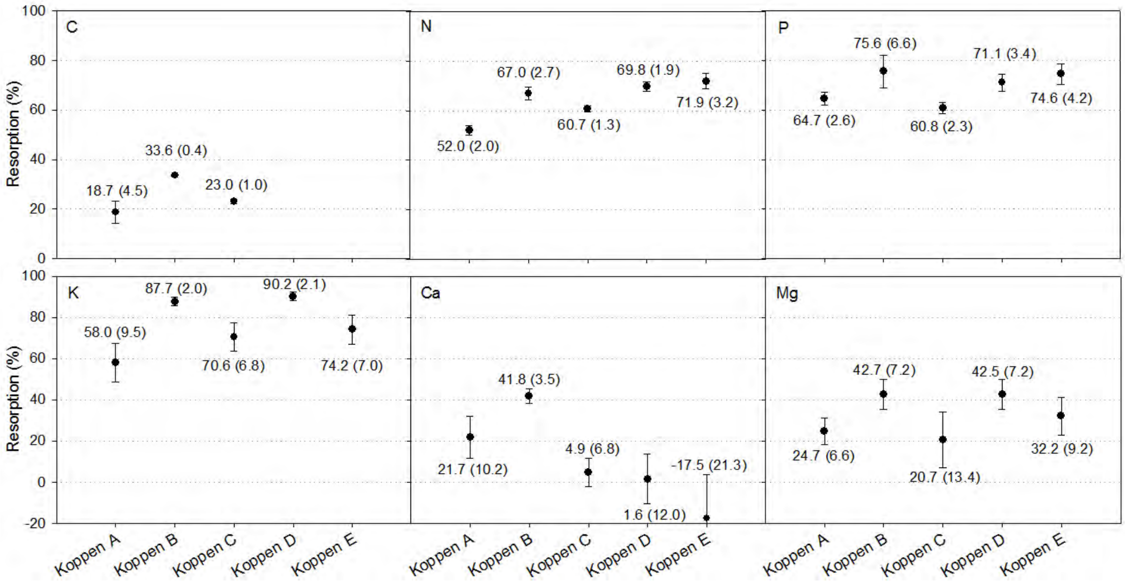

Figure 6. Mean nutrient resorption by climate for all nutrients studied. Values under or above symbols are the mean nutrient resorption for each given point. Within parentheses are the 95% confidence intervals. Koppen's groups are described in detail in the Methods, with: A – Tropical; B – Dry (arid and semiarid) climates); C – Temperate climates; D – Continental and; E – Polar climates.

Among the studied climatic groups, dry climates (Koppen B) tended to have the greatest values $\overline{\text{NuR}}$ for most nutrients, while tropical climates (Koppen A) had the lowest $\overline{\text{NuR}}$, except for Ca (Figure 6). Usually, $\overline{\text{NuR}}$ correlated positively with latitude and negatively with MAT and MAP (except again for $\overline{\text{CaR}}$; see details in the APPENDIX). Latitude, MAT, and MAP also showed strong and statistically significant correlations with green leaf weight (-0.66, 0.62, and 0.57, respectively; *P*<0.001), which in turn correlated negatively with $\overline{\text{NR}}$, $\overline{\text{KR}}$, $\overline{\text{CaR}}$, and $\overline{\text{MgR}}$ (-0.27 (*P*<0.05), -0.81 (*P*<0.001), -0.47 (*P*<0.05), and -0.86 (*P*<0.001), respectively; see APPENDIX).

Table 6 compares β exponents among climates. Temperate and continental climates (Koppen C and D, respectively) showed $\beta>1$ for all nutrients, except Mg. In contrast, tropical, dry, and polar climates (Koppen A, B, and E, respectively) showed $\beta>1$ only for N and P resorption ($P<0.05$). The high β value of 2.54 for P resorption in dry climates (Koppen B) shows that $\overline{\mathrm{PR}}$ depends strongly on green leaves P status in this environment.

4. DISCUSSION

4.1 Nutrient-resorption efficiency patterns

4.1.1 Mass loss during senescence

Our results provide new insights for C, N, and P resorption that build upon earlier work (Aerts 1996, Kobe et al. 2005, Yuan and Chen 2009a, b) and that provide to our knowledge the first global estimates for K, Ca, and Mg resorption. Unlike the often cited 50% $\overline{\mathrm{NR}}$ and $\overline{\mathrm{PR}}$ found in the literature (Aerts 1996, Yuan et al. 2005, Huang et al. 2007, Yuan and Chen 2009a, Kilic et al. 2010), we estimate that $\overline{\mathrm{NR}}$ and $\overline{\mathrm{PR}}$ for terrestrial plants in general are 62.1% and 64.9%, respectively (Figure 3). The major difference between our estimates and this former 50% value arise from the fact that we took into account mass loss (and thus the change in measurement basis) that occurs during senescence. Ignoring this leads to an underestimation of nutrient resorption of ~10% (van Heerwaarden et al. 2003). Mass loss can be caused by several processes, including leaching and translocation before abscission, and is affected by different environmental factors such as rainfall, temperature, and nutrient status of a plant. Here we discuss how such diverse factors might affect mass loss and consequently resorption estimates.

Leaching is present throughout the life spans of all plants, carrying organic and inorganic compounds out of leaves and other organs. Of the inorganic nutrients leached from plants, K, Ca and Mg are usually leached in the greatest quantities (Tukey Jr. 1970). Despite the potential importance of leaching, especially in wetter climates, there is fairly strong evidence showing that leaching has very little influence on leaf nutrient pool depletion during senescence, at least for N and P (Freschet et al. 2010). One of the reasons why leaching appears to affect nutrient absorption estimates only marginally is the short duration of senescence and abscission, which does not allow substantial leaching to occur. If leaching played a prominent role in nutrient resorption during senescence, we would expect nutrient resorption to show a positive correlation with mean annual precipitation. In contrast, our data show no correlation between mean annual precipitation and $\overline{\mathrm{CaR}}$ and $\overline{\mathrm{MgR}}$ and this correlation was even negative for $\overline{\mathrm{CR}}$, $\overline{\mathrm{NR}}$, $\overline{\mathrm{PR}}$, and $\overline{\mathrm{KR}}$ (APPENDIX). This result lends further support to the hypothesis that leaching is not significantly affecting resorption efficiency of the nutrients, at least at the large scales we are investigating.

Besides leaching and resorption, mass loss may also be caused by decomposition and photo-degradation. Even high quality, nutrient-rich leaves, which can be easily degraded by decomposers, generally require at least two weeks under ideal environmental conditions for leaf mass loss to start occurring (Palm and Sanchez 1990, Berg and McClaugherty 2007). Photochemical mineralization of organic material can be an important factor that controls carbon turnover, causing loss of up to 60% of the whole mass in water-limited ecosystems (Austin and Vivanco 2006). Similarly to in-situ decomposition, however, photo-degradation is a slow process when compared to leaf senescence and abscission. As a consequence, if decomposition and/or photo-degradation take place on the leaf mass loss during senescence, their contribution should be small, given the frame time of senescence.

Based on our analysis, C resorption can instead contribute most of the leaf mass loss during senescence. Mean C resorption >30% in herbaceous species and ~20% in trees in fact compares well with 30-35% and 20-25% LML in the same broad plant groups.

4.1.2 Nutrient content and resorption efficiency for plant functional types

During their evolutionary histories, evergreen and deciduous species likely differentiated in part as a consequence of water availability. Shedding of leaves in deciduous species can be partly explained as an adaptation to climates with a marked dry season (Axelrod 1966). However, since a negative correlation between the predominance of evergreens and soil fertility was first documented (Monk 1966), numerous papers have discussed the adaptive significance of evergreens in low-nutrient conditions (Chapin 1980, Aerts 1990, 1996, Killingbeck 1996, Lambers et al. 1998, Yuan and Chen 2009a). Nutrient concentrations and resorption efficiencies can be used to assess if indeed evergreens developed tighter nutrient conservation strategies to cope with these environments. Aerts (1996) found that N resorption was significantly lower in evergreen species and forbs than in deciduous and graminoids. Recently, Yuan & Chen (2009a) also showed that N resorption is lower in evergreen than in deciduous species, but not P resorption. According to Kobe et al. (2005), evergreens and deciduous trees share common functional relationships between senesced and green leaf nutrient contents. Other studies have observed more consistent differences in leaf traits between evergreen and. deciduous species, although such analyses may also include systematic differences in leaf life-spans (e.g., Reich et al. 1992, Hobbie and Gough 2002). We found that nutrient contents in green and senesced leaves differed between evergreen and deciduous species only for C_{Sen}, N_{Gr}, P_{Gr}, and P_{Sen} (Table 2), which were higher in deciduous species. For nutrient resorption, deciduous species showed higher $\overline{CR}$, $\overline{NR}$, and $\overline{KR}$ compared to evergreens, with no differences for $\overline{PR}$, $\overline{CaR}$, and $\overline{MgR}$. Our results confirm that, at the global scale we are investigating, there are indeed important differences in nutrient concentrations and resorption, but that these differences are not universal.

Although Aerts (1996) concluded that there were at best minor differences in nutrient resorption efficiency between plant growth types, our results show that these differences can be large, as in the case of K, for which graminoids showed 84.9% $\overline{\text{KR}}$ and evergreen woody angiosperms 56.1% $\overline{\text{KR}}$ (Figure 4). In fact, graminoids had among the highest resorptions of all nutrients examined, followed by conifers and forbs. The largest differences observed were for N, P, and K, where graminoids had 74.6% $\overline{\text{NR}}$, 82.1% $\overline{\text{PR}}$, and 84.9% $\overline{\text{KR}}$, much higher than the average of 50% typically assumed in most terrestrial models for N and P (Table 1) and reported by Yuan & Chen (2009a) for both conifers and broadleaf species.

Ca is known to be a structural element in plants, particularly in cell walls, that tends to be resorbed little during senescence (Tukey Jr. 1970, Lambers et al. 1998, Kazakou et al. 2007). Previous research has suggested that Ca is generally conserved in leaves (van Heerwaarden et al. 2003). In contrast, our data showed, on average, a 10.9% $\overline{\text{CaR}}$ during senescence (Figure 3). While conifers and deciduous and evergreen woody angiosperms showed a $\overline{\text{CaR}}$ statistically indistinguishable from zero, forbs and graminoids showed 36.9 and 32.5% $\overline{\text{CaR}}$, respectively (Figure 4). Hence, these results show that Ca is not a sufficiently conservative measurement basis and thus should not be used to estimate unbiased resorption efficiencies.

4.1.3 Resorption efficiency and nutrient status

Although correlations between $\overline{\text{NuR}}$ and nutrient status have not typically been found in previous studies (Chapin and Moilanen 1991, Aerts 1996, Lambers et al. 1998, Aerts and Chapin 2000, Kazakou et al. 2007, Yuan and Chen 2009a), a study by Kobe et al. (2005) showed that N and P resorption efficiencies generally declined with increasing leaf nutrient status. Our power-law regression analysis confirmed that C, N, P, K, and Mg resorption are more efficient in green leaves that have low nutrient concentrations (β>1) and showed that Ca resorption is independent of Ca status (β=1; Table 5 and Figure 5). From the perspective of leaf economics, this pattern of more efficient resorption at low nutrient concentrations is expected, corroborating the notion that plants will use the least energetically costly process to acquire nutrients.

Overall C resorption had β>1, suggesting increased C resorption in C-poor leaves (Tables 5 and 6). If relative changes in leaf C concentrations were mainly due to different concentration of non-structural carbohydrates (NSC), C resorption would be expected to be correlated to NSC content. Also, C resorption should show β>1 for all plant types, as NSC are relatively easily resorbed. Concentrations of NSC have been found to decrease with mean growing-season temperature, ranging from treeline conifers (NSC ~18% dry mass, 8 ^{o}C) to tropical lowlands (~6% NSC; 27 ^{o}C), with an average value for trees of ~11% (Körner 2003). Herbaceous plants and shrubs show an average of 15.3% NSC content in leaves. An upper limit of NSC content in leaves was found to be 23% (Körner and Miglietta 1994). If NSC were the main C compounds being resorbed, this upper NSC value of ~25% should mirror the upper theoretical limit for C resorption. Given that starch ($C_6H_{10}O_5$) and sugars ($C_6H_{12}O_6$) are the main NSC compounds in leaves (Taiz and Zeiger 2002), and they have an average C concentration of 42%, an upper limit of 25% NSC results in a potential maximum ~10% C resorption. Our data show an average C resorption of 23.2% and a β value slightly larger than one, thus corroborating the hypothesis that NSC is responsible for less than 50% of C resorption.

For N and P resorption, β>1 held for all plant types and climates, suggesting that resorption increases in relatively N- and P-poor leaves globally. For the other nutrients, β values varied among plant types and climates either being equal to or greater than one. In only one case did β reach a negative value – Mg resorption in continental climates. From a leaf economics perspective, all nutrients for which uptake from the soil is more energy-demanding than resorption would be expected to show $\beta > 1$.

4.1.4 Resorption efficiency and climate

Our analysis contrasts with some of the conclusions of Yuan & Chen (2009a), who found that N resorption increased with latitude but decreased with MAT and MAP, with P resorption showing opposite relationships. We found significant and similar, rather than contrasting, climate trends for N and P. Both nutrients correlated negatively with MAT and MAP, and positively with latitude (APPENDIX). This pattern of negative relationships between $\overline{\text{NuR}}$ and MAT and MAP, and a positive relationship between $\overline{\text{NuR}}$ and latitude, was found in almost all climates and growth types.

Regarding climate and soil fertility, tropical soils are generally considered to be older and relatively lower in fertility than are soils in most other regions. From the standpoint of leaf economics, the low nutrient availability of tropical soils, especially for P availability, would be expected to lead to a generally higher $\overline{\text{NuR}}$, and improved ecosystem nutrient recycling in general (Vitousek 1984, Aerts 1997, Aerts and Chapin 2000, McGroddy et al. 2004). However, as observed in other studies (Reich et al. 1995, Aerts 1996, Diehl et al. 2003), our results do not necessarily show high $\overline{\text{NuR}}$ in low-fertility soils (tropical climate in this case, see Figure 6). In fact, tropical sites (Koppen A) were always among the climates with the lowest $\overline{\text{NuR}}$, except for Ca. This lack of correlation may be due to strong heterogeneity in tropical soils (Richter and Babbar 1991) that leads to a range of nutrient conditions, or to that fact that plants in the tropics have adopted other nutrient conservation strategies (Section 4.2).

4.2 Ecological consequences of observed patterns in resorption efficiency

Source-sink interactions within a plant may strongly influence nutrient resorption (Nambiar and Fife 1987, Chapin and Moilanen 1991, Buchanan-Wollaston 1997, Watson and Lu 2004). Leaf senescence could be repressed, or even reversed, when developing sinks are removed (Crafts-Brandner 1991). When leaf senescence occurs relatively early in the growing season, mineral nutrients, particularly N, can be reallocated to other simultaneous growth processes (stronger sinks and more resorption), but at the expense of potential C acquisition by those leaves. Conversely, when leaves senesce later in the season, the period for assimilate acquisition is longer, at the expense of nutrient reallocation to other developing structures (weaker sinks, less resorption). By having an active growth of new leaves (a strong sink) at the time of leaf senescence, both graminoids and evergreens were predicted to have relatively high nutrient resorption efficiency (Lambers et al. 1998). This explains why graminoids showed the highest $\overline{\text{NuR}}$, although evergreens did not (Figure 4). Beside graminoids, our results showed that only conifers similarly showed such high $\overline{\text{NuR}}$ values, at least for P, K, and Mg.

According to Lambers et al. (1998), nutrient conservation strategies differ between broad-leaved deciduous and conifer species. Conifers typically have lower nutrient concentrations in leaves and relatively higher investments in C-rich compounds, while broad-leaved deciduous species tend to be characterized by higher N, P, and K concentrations in mature leaves and higher N resorption (Lambers et al. 1998). We found that conifer species indeed have lower nutrient concentrations in leaves and differed from angiosperms in leaf nutrient concentration and $\overline{\text{NuR}}$ parameters (Figure 4 and Table 2). However, we did not find evergreen woody angiosperms to be intermediate between conifers and deciduous species, as suggested by Lambers et al. (1998). In fact, evergreen and deciduous woody angiosperms showed very similar nutrient concentrations in leaves and very similar $\overline{\mathrm{NuR}}$ values. The difference between the two groups was in C, N, and K resorption ($P<0.05$), which was significantly higher for deciduous (23.9, 61.2, and 69.2% $\overline{\mathrm{NuR}}$, respectively) than for evergreen species (20.8, 56.1, and 56.1% $\overline{\mathrm{NuR}}$, respectively).

A simple model to explain the dominance of low-productive perennials in nutrient-poor habitats over high-productive perennials has been proposed (Aerts and van der Peijl 1993). They showed that, in nutrient-poor environments, nutrient conserving species can have a higher equilibrium biomass than species with higher nutrient losses. This means that nutrient conservation strategies like high resorption at senescence lead to a direct advantage in nutrient-poor environments. Our data show that graminoids would be the species best adapted to nutrient-poor environments, followed by conifers and forbs, and lastly by evergreen and deciduous woody angiosperms.

Conservation strategies different from improved resorption efficiency might also be important (Hobbie 1992), and could partly explain the lack of correlation between NuR and climatic and soil nutrient conditions. For example, increased leaf lifespan and lower nutrient concentrations (e.g., Aerts and Chapin 2000) could both contribute to higher overall nutrient use efficiency.

4.3 Implications for terrestrial ecosystem modeling

The $\overline{\mathrm{NuR}}$ estimates we obtained should be useful for parameterizing and improving ecosystem and biogeochemical models. $\overline{\mathrm{NuR}}$ plays a major role in determining nutrient concentrations in litter. In turn, litter nutrient concentration controls decomposition rate (Aerts 1997, Berg and McClaugherty 2007) and the trajectories of carbon-to-nutrient ratios during decomposition (Parton et al. 2007, Manzoni et al. 2008, Manzoni et al. 2010), thus affecting nutrient mineralization.

Because the quality of leaves in senescence, the final step of nutrient cycling at the leaf level, is the initial condition for litter decomposition, our $\overline{\mathrm{NuR}}$ parameters provide important information for bridging plant and soil carbon and nutrient cycling.

In the broader context of ecosystem models, nutrient resorption is also a key parameter for defining nutrient requirements and litter quality, with feedbacks to all components of such models. Because N is considered a limiting nutrient for many terrestrial ecosystems, most ecosystem models consider N alone, with a few models also describing P dynamics explicitly (Parton et al. 1993, Wang et al. 2007). In general, the greater the resorption efficiency used in a model, the greater the amount of a nutrient stored for growth in the following year. Thus, prescribing accurate $\overline{\mathrm{NuR}}$ values for models is important for predicting the temporal changes in biomass under limiting nutrient conditions. In some models, resorption efficiency is assumed to be constant for a given species or functional group (Table 1), with values obtained from specific observations or published datasets. In other models, resorption efficiency varies depending on leaf nutrient status, which in turn results from plant stoichiometric constraints and nutrient availability during the growing season, as well as prescribed nutrient concentrations in litter (see Table 1). In other words, resorption efficiency is not defined *a priori* as an intrinsic plant property but instead changes dynamically to accommodate constant nutrient conditions in the litter. This representation is not always physiologically (and mechanistically) meaningful, because the plant does control litter quality by changing its resorption efficiency. Moreover, in this way the nutrient status of the plant might improve in time in spite of limited available soil nutrients, precisely because nutrient losses during senescence are too low. As a consequence, nutrient limitation effects could be underestimated.

5. CONCLUSIONS

Our newly assembled database of nutrient contents in green and senesced leaves worldwide allowed us to compute resorption efficiencies across plant types and climates, accounting for mass loss during senescence. We show here a consistent pattern of leaf mass loss during senescence in terrestrial plants and how neglecting it can lead to an underestimation of nutrient resorption. Based on our global database, overall C, N, P, K, and Mg resorption is nutrient-status dependent, with relatively more nutrients resorbed for lower leaf nutrient concentrations, in agreement with a leaf economics perspective. Overall, our new global estimates for nutrient concentrations and resorption efficiencies should improve models that explicitly represent the cycling of C and nutrients, particularly N and P. They should also allow the modeling community to represent more explicitly the coupling of other nutrient cycles within plants and ecosystems.

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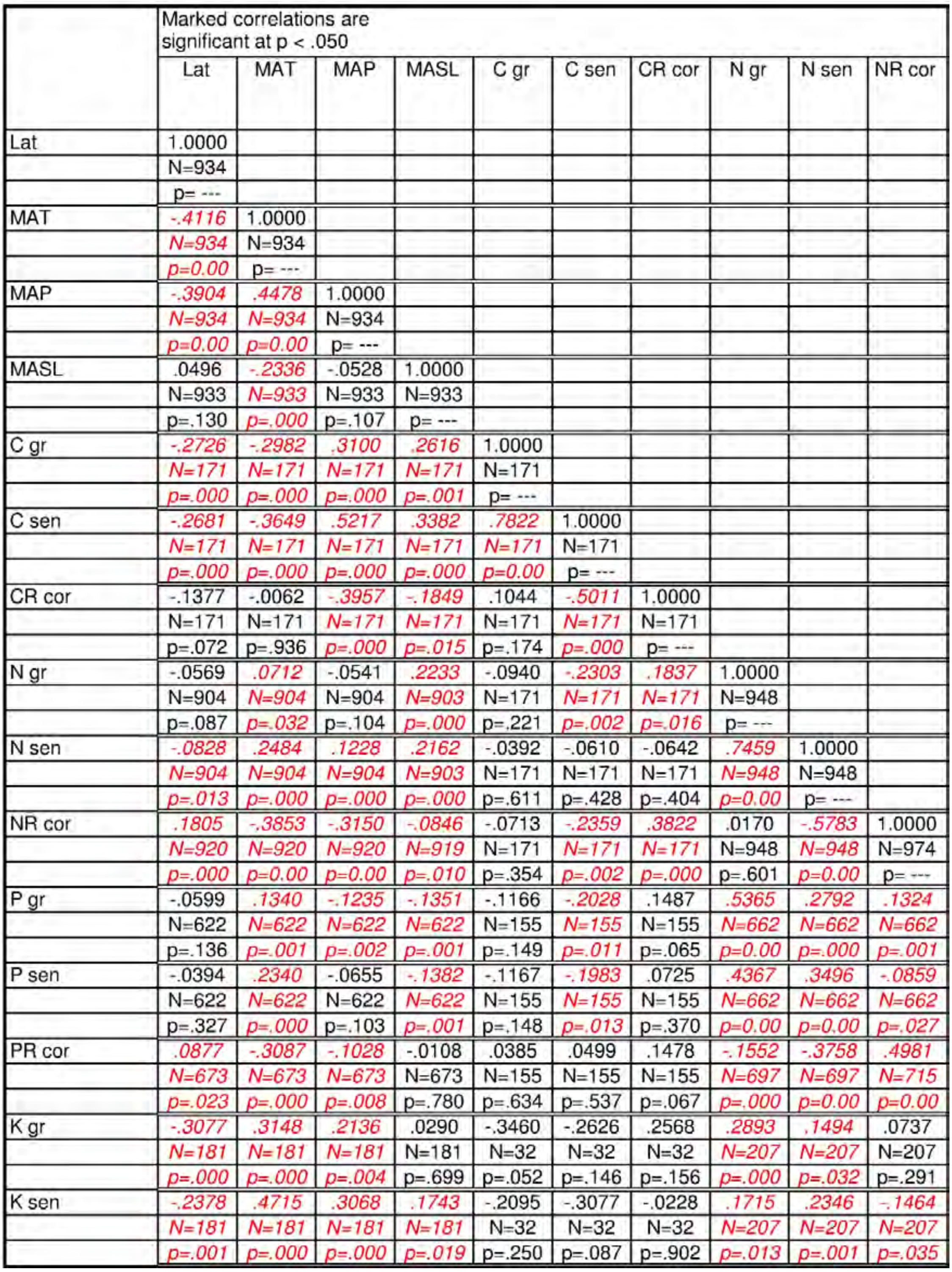
8. APPENDIX

Table A1. Number of datapoints.

	C	N	P	K	Ca	Mg	LML
Total	171	948	669	207	150	115	191
Conifers	4	81	53	30	15	15	24
Ferns	0	22	22	0	0	22	0
Forbs	0	88	58	24	22	0	18
Graminoids	15	83	51	15	16	12	18
Dec. ang.	75	363	260	89	54	30	63
Ev. ang.	77	302	218	49	43	36	68
Koppen A	12	177	154	49	47	44	-
Koppen B	8	76	13	8	8	8	-
Koppen C	149	484	351	84	66	34	-
Koppen D	2	165	110	53	16	16	-
Koppen E	0	29	27	13	13	13	-

Table A2. Pearson’s correlation.

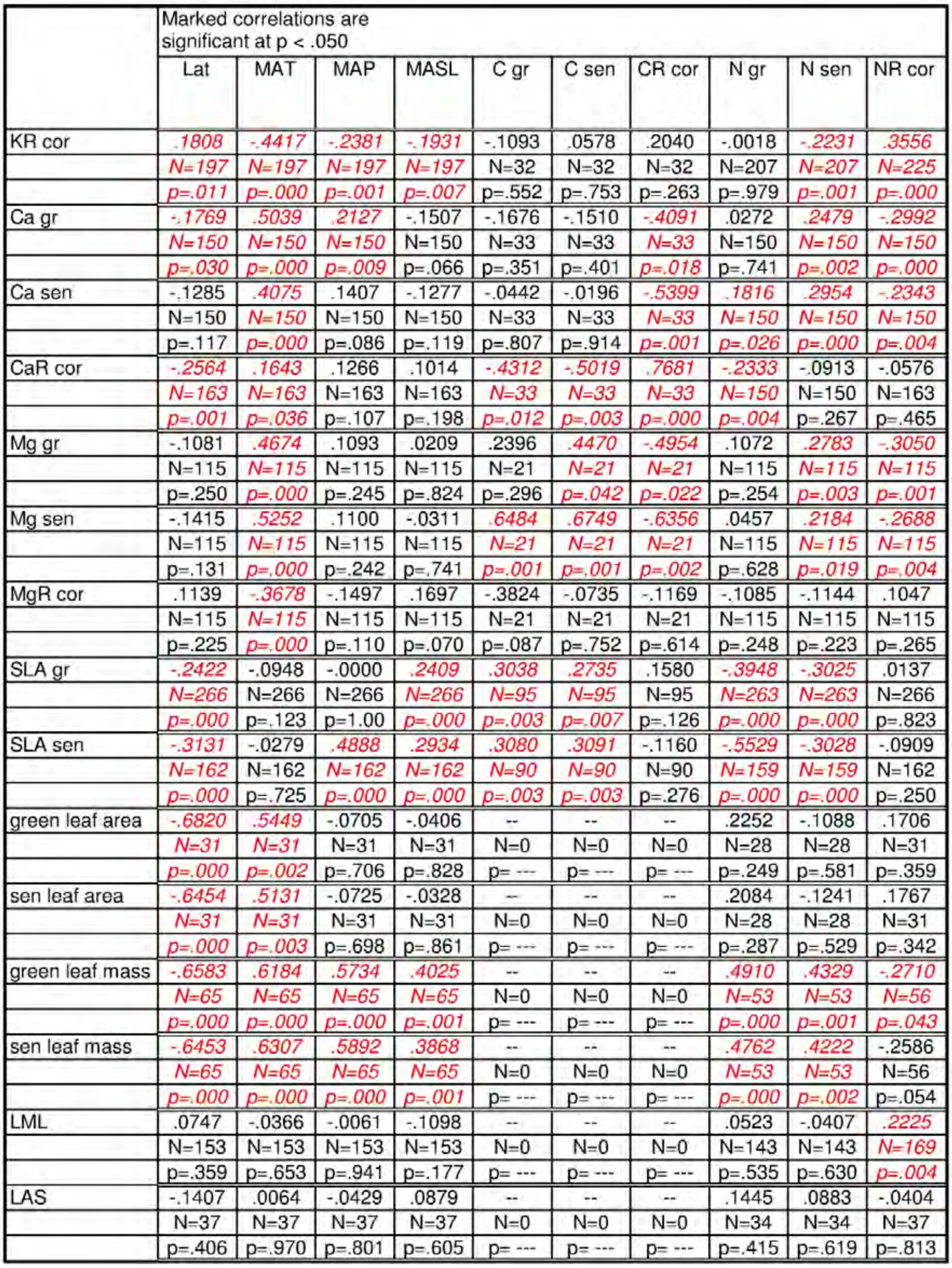


	Marked correlations are significant at p < .050									
	Lat	MAT	MAP	MASL	C gr	C sen	CR cor	N gr	N sen	NR cor
Lat	1.0000									
	N=934									
	p= ---									
MAT	*-.4116*	1.0000								
	N=934	N=934								
	p=0.00	p= ---								
MAP	*-.3904*	*.4478*	1.0000							
	N=934	*N=934*	N=934							
	p=0.00	*p=0.00*	p= ---							
MASL	.0496	*-.2336*	-.0528	1.0000						
	N=933	*N=933*	N=933	N=933						
	p=.130	*p=.000*	p=.107	p= ---						
C gr	*-.2726*	*-.2982*	*.3100*	*.2616*	1.0000					
	N=171	*N=171*	*N=171*	*N=171*	N=171					
	p=.000	*p=.000*	*p=.000*	*p=.001*	p= ---					
C sen	*-.2681*	*-.3649*	*.5217*	*.3382*	*.7822*	1.0000				
	N=171	*N=171*	*N=171*	*N=171*	*N=171*	N=171				
	p=.000	*p=.000*	*p=.000*	*p=.000*	*p=0.00*	p= ---				
CR cor	-.1377	-.0062	*-.3957*	*-.1849*	.1044	*-.5011*	1.0000			
	N=171	N=171	*N=171*	*N=171*	N=171	*N=171*	N=171			
	p=.072	p=.936	*p=.000*	*p=.015*	p=.174	*p=.000*	p= ---			
N gr	-.0569	*.0712*	-.0541	*.2233*	-.0940	*-.2303*	*.1837*	1.0000		
	N=904	*N=904*	N=904	*N=903*	N=171	*N=171*	*N=171*	N=948		
	p=.087	*p=.032*	p=.104	*p=.000*	p=.221	*p=.002*	*p=.016*	p= ---		
N sen	*-.0828*	*.2484*	*.1228*	*.2162*	-.0392	-.0610	-.0642	*.7459*	1.0000	
	N=904	*N=904*	*N=904*	*N=903*	N=171	N=171	N=171	*N=948*	N=948	
	p=.013	*p=.000*	*p=.000*	*p=.000*	p=.611	p=.428	p=.404	*p=0.00*	p= ---	
NR cor	*.1805*	*-.3853*	*-.3150*	*-.0846*	-.0713	*-.2359*	*.3822*	.0170	*-.5783*	1.0000
	N=920	*N=920*	*N=920*	*N=919*	N=171	*N=171*	*N=171*	N=948	*N=948*	N=974
	p=.000	*p=0.00*	*p=0.00*	*p=.010*	p=.354	*p=.002*	*p=.000*	p=.601	*p=0.00*	p= ---
P gr	-.0599	*.1340*	*-.1235*	*-.1351*	-.1166	*-.2028*	.1487	*.5365*	*.2792*	*.1324*
	N=622	*N=622*	*N=622*	*N=622*	N=155	*N=155*	N=155	*N=662*	*N=662*	*N=662*
	p=.136	*p=.001*	*p=.002*	*p=.001*	p=.149	*p=.011*	p=.065	*p=0.00*	*p=.000*	*p=.001*
P sen	-.0394	*.2340*	-.0655	*-.1382*	-.1167	*-.1983*	.0725	*.4367*	*.3496*	*-.0859*
	N=622	*N=622*	N=622	*N=622*	N=155	*N=155*	N=155	*N=662*	*N=662*	*N=662*
	p=.327	*p=.000*	p=.103	*p=.001*	p=.148	*p=.013*	p=.370	*p=0.00*	*p=0.00*	*p=.027*
PR cor	*.0877*	*-.3087*	*-.1028*	-.0108	.0385	.0499	.1478	*-.1552*	*-.3758*	*.4981*
	N=673	*N=673*	*N=673*	N=673	N=155	N=155	N=155	*N=697*	*N=697*	*N=715*
	p=.023	*p=.000*	*p=.008*	p=.780	p=.634	p=.537	p=.067	*p=.000*	*p=0.00*	*p=0.00*
K gr	*-.3077*	*.3148*	*.2136*	.0290	-.3460	-.2626	.2568	*.2893*	*.1494*	.0737
	N=181	*N=181*	*N=181*	N=181	N=32	N=32	N=32	*N=207*	*N=207*	N=207
	p=.000	*p=.000*	*p=.004*	p=.699	p=.052	p=.146	p=.156	*p=.000*	*p=.032*	p=.291
K sen	*-.2378*	*.4715*	*.3068*	*.1743*	-.2095	-.3077	-.0228	*.1715*	*.2346*	*-.1464*
	N=181	*N=181*	*N=181*	*N=181*	N=32	N=32	N=32	*N=207*	*N=207*	*N=207*
	p=.001	*p=.000*	*p=.000*	*p=.019*	p=.250	p=.087	p=.902	*p=.013*	*p=.001*	*p=.035*

...continuing Table A2

	Marked correlations are significant at p < .050									
	P gr	P sen	PR cor	K gr	K sen	KR cor	Ca gr	Ca sen	CaR cor	Mg gr
Lat										
MAT										
MAP										
MASL										
C gr										
C sen										
CR cor										
N gr										
N sen										
NR cor										
P gr	1.0000									
	N=669									
	p= ---									
P sen	*.8222*	1.0000								
	N=669	N=669								
	p=0.00	p= ---								
PR cor	*-.1611*	*-.5583*	1.0000							
	N=669	*N=669*	N=722							
	p=.000	*p=0.00*	p= ---							
K gr	*.4146*	*.1520*	.0571	1.0000						
	N=204	*N=204*	N=204	N=207						
	p=.000	*p=.030*	p=.417	p= ---						
K sen	*.2204*	*.2026*	*-.1378*	*.7503*	1.0000					
	N=204	*N=204*	*N=204*	*N=207*	N=207					
	p=.002	*p=.004*	*p=.049*	*p=0.00*	p= ---					

...continuing Table A2



	Marked correlations are significant at p < .050									
	Lat	MAT	MAP	MASL	C gr	C sen	CR cor	N gr	N sen	NR cor
KR cor	*.1808*	*-.4417*	*-.2381*	*-.1931*	-.1093	.0578	.2040	-.0018	*-.2231*	*.3556*
	N=197	*N=197*	*N=197*	*N=197*	N=32	N=32	N=32	N=207	*N=207*	*N=225*
	p=.011	*p=.000*	*p=.001*	*p=.007*	p=.552	p=.753	p=.263	p=.979	*p=.001*	*p=.000*
Ca gr	*-.1769*	*.5039*	*.2127*	-.1507	-.1676	-.1510	*-.4091*	.0272	*.2479*	*-.2992*
	N=150	*N=150*	*N=150*	N=150	N=33	N=33	*N=33*	N=150	*N=150*	*N=150*
	p=.030	*p=.000*	*p=.009*	p=.066	p=.351	p=.401	*p=.018*	p=.741	*p=.002*	*p=.000*
Ca sen	-.1285	*.4075*	.1407	-.1277	-.0442	-.0196	*-.5399*	*.1816*	*.2954*	*-.2343*
	N=150	*N=150*	N=150	N=150	N=33	N=33	*N=33*	*N=150*	*N=150*	*N=150*
	p=.117	*p=.000*	p=.086	p=.119	p=.807	p=.914	*p=.001*	*p=.026*	*p=.000*	*p=.004*
CaR cor	*-.2564*	*.1643*	.1266	.1014	*-.4312*	*-.5019*	*.7681*	*-.2333*	-.0913	-.0576
	N=163	*N=163*	N=163	N=163	*N=33*	*N=33*	*N=33*	*N=150*	N=150	N=163
	p=.001	*p=.036*	p=.107	p=.198	*p=.012*	*p=.003*	*p=.000*	*p=.004*	p=.267	p=.465
Mg gr	-.1081	*.4674*	.1093	.0209	.2396	*.4470*	*-.4954*	.1072	*.2783*	*-.3050*
	N=115	*N=115*	N=115	N=115	N=21	*N=21*	*N=21*	N=115	*N=115*	*N=115*
	p=.250	*p=.000*	p=.245	p=.824	p=.296	*p=.042*	*p=.022*	p=.254	*p=.003*	*p=.001*
Mg sen	-.1415	*.5252*	.1100	-.0311	*.6484*	*.6749*	*-.6356*	.0457	*.2184*	*-.2688*
	N=115	*N=115*	N=115	N=115	*N=21*	*N=21*	*N=21*	N=115	*N=115*	*N=115*
	p=.131	*p=.000*	p=.242	p=.741	*p=.001*	*p=.001*	*p=.002*	p=.628	*p=.019*	*p=.004*
MgR cor	.1139	*-.3678*	-.1497	.1697	-.3824	-.0735	-.1169	-.1085	-.1144	.1047
	N=115	*N=115*	N=115	N=115	N=21	N=21	N=21	N=115	N=115	N=115
	p=.225	*p=.000*	p=.110	p=.070	p=.087	p=.752	p=.614	p=.248	p=.223	p=.265
SLA gr	*-.2422*	-.0948	-.0000	*.2409*	*.3038*	*.2735*	.1580	*-.3948*	*-.3025*	.0137
	N=266	N=266	N=266	*N=266*	*N=95*	*N=95*	N=95	*N=263*	*N=263*	N=266
	p=.000	p=.123	p=1.00	*p=.000*	*p=.003*	*p=.007*	p=.126	*p=.000*	*p=.000*	p=.823
SLA sen	*-.3131*	-.0279	*.4888*	*.2934*	*.3080*	*.3091*	-.1160	*-.5529*	*-.3028*	-.0909
	N=162	N=162	*N=162*	*N=162*	*N=90*	*N=90*	N=90	*N=159*	*N=159*	N=162
	p=.000	p=.725	*p=.000*	*p=.000*	*p=.003*	*p=.003*	p=.276	*p=.000*	*p=.000*	p=.250
green leaf area	*-.6820*	*.5449*	-.0705	-.0406	--	--	--	.2252	-.1088	.1706
	N=31	*N=31*	N=31	N=31	N=0	N=0	N=0	N=28	N=28	N=31
	p=.000	*p=.002*	p=.706	p=.828	p= ---	p= ---	p= ---	p=.249	p=.581	p=.359
sen leaf area	*-.6454*	*.5131*	-.0725	-.0328	--	--	--	.2084	-.1241	.1767
	N=31	*N=31*	N=31	N=31	N=0	N=0	N=0	N=28	N=28	N=31
	p=.000	*p=.003*	p=.698	p=.861	p= ---	p= ---	p= ---	p=.287	p=.529	p=.342
green leaf mass	*-.6583*	*.6184*	*.5734*	*.4025*	--	--	--	*.4910*	*.4329*	*-.2710*
	N=65	*N=65*	*N=65*	*N=65*	N=0	N=0	N=0	*N=53*	*N=53*	*N=56*
	p=.000	*p=.000*	*p=.000*	*p=.001*	p= ---	p= ---	p= ---	*p=.000*	*p=.001*	*p=.043*
sen leaf mass	*-.6453*	*.6307*	*.5892*	*.3868*	--	--	--	*.4762*	*.4222*	-.2586
	N=65	*N=65*	*N=65*	*N=65*	N=0	N=0	N=0	*N=53*	*N=53*	N=56
	p=.000	*p=.000*	*p=.000*	*p=.001*	p= ---	p= ---	p= ---	*p=.000*	*p=.002*	p=.054
LML	.0747	-.0366	-.0061	-.1098	--	--	--	.0523	-.0407	*.2225*
	N=153	N=153	N=153	N=153	N=0	N=0	N=0	N=143	N=143	*N=169*
	p=.359	p=.653	p=.941	p=.177	p= ---	p= ---	p= ---	p=.535	p=.630	*p=.004*
LAS	-.1407	.0064	-.0429	.0879	--	--	--	.1445	.0883	-.0404
	N=37	N=37	N=37	N=37	N=0	N=0	N=0	N=34	N=34	N=37
	p=.406	p=.970	p=.801	p=.605	p= ---	p= ---	p= ---	p=.415	p=.619	p=.813

...continuing Table A2

	Marked correlations are significant at p < .050									
	P gr	P sen	PR cor	K gr	K sen	KR cor	Ca gr	Ca sen	CaR cor	Mg gr
KR cor	.1014	*-.2188*	*.3881*	.0270	*-.4330*	1.0000				
	N=204	*N=204*	*N=222*	N=207	*N=207*	N=225				
	p=.149	*p=.002*	*p=.000*	p=.699	*p=.000*	p= ---				
Ca gr	-.0347	*.2489*	*-.2923*	*.3758*	*.6065*	*-.4998*	1.0000			
	N=147	*N=147*	*N=147*	*N=146*	*N=146*	*N=146*	N=150			
	p=.676	*p=.002*	*p=.000*	*p=.000*	*p=.000*	*p=.000*	p= ---			
Ca sen	.1410	*.3456*	*-.2825*	*.3823*	*.5873*	*-.5020*	*.8491*	1.0000		
	N=147	*N=147*	*N=147*	*N=146*	*N=146*	*N=146*	*N=150*	N=150		
	p=.088	*p=.000*	*p=.001*	*p=.000*	*p=.000*	*p=.000*	*p=0.00*	p= ---		
CaR cor	*-.2243*	-.1400	-.0023	.1550	.1269	.0438	*.2364*	*-.2027*	1.0000	
	N=147	N=147	N=160	N=146	N=146	N=159	*N=150*	*N=150*	N=163	
	p=.006	p=.091	p=.977	p=.062	p=.127	p=.583	*p=.004*	*p=.013*	p= ---	
Mg gr	-.0589	*.2193*	*-.4618*	*.2059*	*.3443*	*-.5681*	*.6327*	*.5248*	*.1864*	1.0000
	N=113	*N=113*	*N=113*	*N=115*	*N=115*	*N=115*	*N=114*	*N=114*	*N=114*	N=115
	p=.535	*p=.020*	*p=.000*	*p=.027*	*p=.000*	*p=.000*	*p=.000*	*p=.000*	*p=.047*	p= ---
Mg sen	-.0606	*.2155*	*-.4768*	*.2203*	*.4186*	*-.6690*	*.6414*	*.6047*	.1095	*.8983*
	N=113	*N=113*	*N=113*	*N=115*	*N=115*	*N=115*	*N=114*	*N=114*	N=114	*N=115*
	p=.524	*p=.022*	*p=.000*	*p=.018*	*p=.000*	*p=.000*	*p=.000*	*p=.000*	p=.246	*p=0.00*
MgR cor	-.1451	*-.3004*	*.3351*	-.0094	-.1529	*.4056*	-.0998	*-.2907*	*.3001*	-.0651
	N=113	*N=113*	*N=113*	N=115	N=115	*N=115*	N=114	*N=114*	*N=114*	N=115
	p=.125	*p=.001*	*p=.000*	p=.921	p=.103	*p=.000*	p=.291	*p=.002*	*p=.001*	p=.489
SLA gr	*-.2669*	*-.2571*	*.1382*	-.3913	*-.5881*	.3306	-.3483	-.2418	*-.6521*	*-.7015*
	N=209	*N=209*	*N=212*	N=21	*N=21*	N=24	N=21	N=21	*N=21*	*N=13*
	p=.000	*p=.000*	*p=.044*	p=.079	*p=.005*	p=.115	p=.122	p=.291	*p=.001*	*p=.008*
SLA sen	*-.3832*	*-.3430*	*.2586*	-.4377	-.5113	.0305	-.4342	-.3907	-.3180	--
	N=129	*N=129*	*N=132*	N=8	N=8	N=11	N=8	N=8	N=8	N=0
	p=.000	*p=.000*	*p=.003*	p=.278	p=.195	p=.929	p=.282	p=.339	p=.443	p= ---
green leaf area	.0976	-.1718	.2753	--	--	.6234	--	--	--	--
	N=26	N=26	N=29	N=0	N=0	N=3	N=0	N=0	N=0	N=0
	p=.635	p=.401	p=.148	p= ---	p= ---	p=.572	p= ---	p= ---	p= ---	p= ---
sen leaf area	.0901	-.1854	.2875	--	--	.5826	--	--	--	--
	N=26	N=26	N=29	N=0	N=0	N=3	N=0	N=0	N=0	N=0
	p=.662	p=.365	p=.131	p= ---	p= ---	p=.604	p= ---	p= ---	p= ---	p= ---
green leaf mass	.0267	.1159	-.2365	*.9530*	*.9775*	*-.8060*	*.9492*	*.9858*	*-.4674*	*.9637*
	N=50	N=50	N=53	*N=24*	*N=24*	*N=27*	*N=24*	*N=24*	*N=24*	*N=24*
	p=.854	p=.423	p=.088	*p=.000*	*p=.000*	*p=.000*	*p=.000*	*p=.000*	*p=.021*	*p=.000*
sen leaf mass	.0382	.1177	-.2273	*.9465*	*.9864*	*-.7847*	*.9013*	*.9906*	*-.5760*	*.9394*
	N=50	N=50	N=53	*N=24*	*N=24*	*N=27*	*N=24*	*N=24*	*N=24*	*N=24*
	p=.792	p=.416	p=.102	*p=.000*	*p=.000*	*p=.000*	*p=.000*	*p=0.00*	*p=.003*	*p=.000*
LML	.0289	-.0654	*.2802*	.0412	-.1588	*.4171*	-.1659	-.3802	*.3334*	-.2774
	N=121	N=121	*N=139*	N=44	N=44	*N=62*	N=24	N=24	*N=37*	N=24
	p=.753	p=.476	*p=.001*	p=.791	p=.303	*p=.001*	p=.438	p=.067	*p=.044*	p=.189
LAS	.0239	.1736	-.1284	--	--	-.0430	--	--	--	--
	N=32	N=32	N=35	N=0	N=0	N=3	N=0	N=0	N=0	N=0
	p=.897	p=.342	p=.462	p= ---	p= ---	p=.973	p= ---	p= ---	p= ---	p= ---

...continuing Table A2

	Marked correlations are significant at p < .050									
	Mg sen	MgR cor	SLA gr	SLA sen	green leaf area	sen leaf area	green leaf mass	sen leaf mass	LML	LAS
KR cor										
Ca gr										
Ca sen										
CaR cor										
Mg gr										
Mg sen	1.0000									
	N=115									
	p= ---									
MgR cor	*-.3941*	1.0000								
	N=115	N=115								
	p=.000	p= ---								
SLA gr	*-.7083*	.0183	1.0000							
	N=13	N=13	N=266							
	p=.007	p=.953	p= ---							
SLA sen	--	--	*.9591*	1.0000						
	N=0	N=0	*N=162*	N=162						
	p= ---	p= ---	*p=0.00*	p= ---						
green leaf area	--	--	-.2703	-.3181	1.0000					
	N=0	N=0	N=31	N=31	N=31					
	p= ---	p= ---	p=.141	p=.081	p= ---					
sen leaf area	--	--	-.2370	-.2866	*.9938*	1.0000				
	N=0	N=0	N=31	N=31	*N=31*	N=31				
	p= ---	p= ---	p=.199	p=.118	*p=0.00*	p= ---				
green leaf mass	*.9912*	*-.8580*	.3183	.2432	*.7466*	*.7711*	1.0000			
	N=24	*N=24*	N=31	N=31	*N=31*	*N=31*	N=65			
	p=0.00	*p=.000*	p=.081	p=.187	*p=.000*	*p=.000*	p= ---			
sen leaf mass	*.9895*	*-.8901*	*.4312*	*.3753*	*.6504*	*.6891*	*.9843*	1.0000		
	N=24	*N=24*	*N=31*	*N=31*	*N=31*	*N=31*	*N=65*	N=65		
	p=0.00	*p=.000*	*p=.015*	*p=.037*	*p=.000*	*p=.000*	*p=0.00*	p= ---		
LML	-.3615	*.4417*	*-.3191*	-.0342	*.3703*	.3031	-.1930	*-.2942*	1.0000	
	N=24	*N=24*	*N=54*	N=36	*N=31*	N=31	N=65	*N=65*	N=191	
	p=.083	*p=.031*	*p=.019*	p=.843	*p=.040*	p=.097	p=.124	*p=.017*	p= ---	
LAS	--	--	*-.3785*	*-.3700*	.1395	.0453	-.1124	-.2419	*.6821*	1.0000
	N=0	N=0	*N=31*	*N=31*	N=31	N=31	N=31	N=31	*N=37*	N=37
	p= ---	p= ---	*p=.036*	*p=.040*	p=.454	p=.809	p=.547	p=.190	*p=.000*	p= ---

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CHAPTER 2

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THERMODYNAMIC CHARACTERIZATION OF HUMIC ACID-SURFACTANT INTERACTION. NEW INSIGHTS ON HUMIC ACIDS' BEHAVIOR AND STRUCTURE

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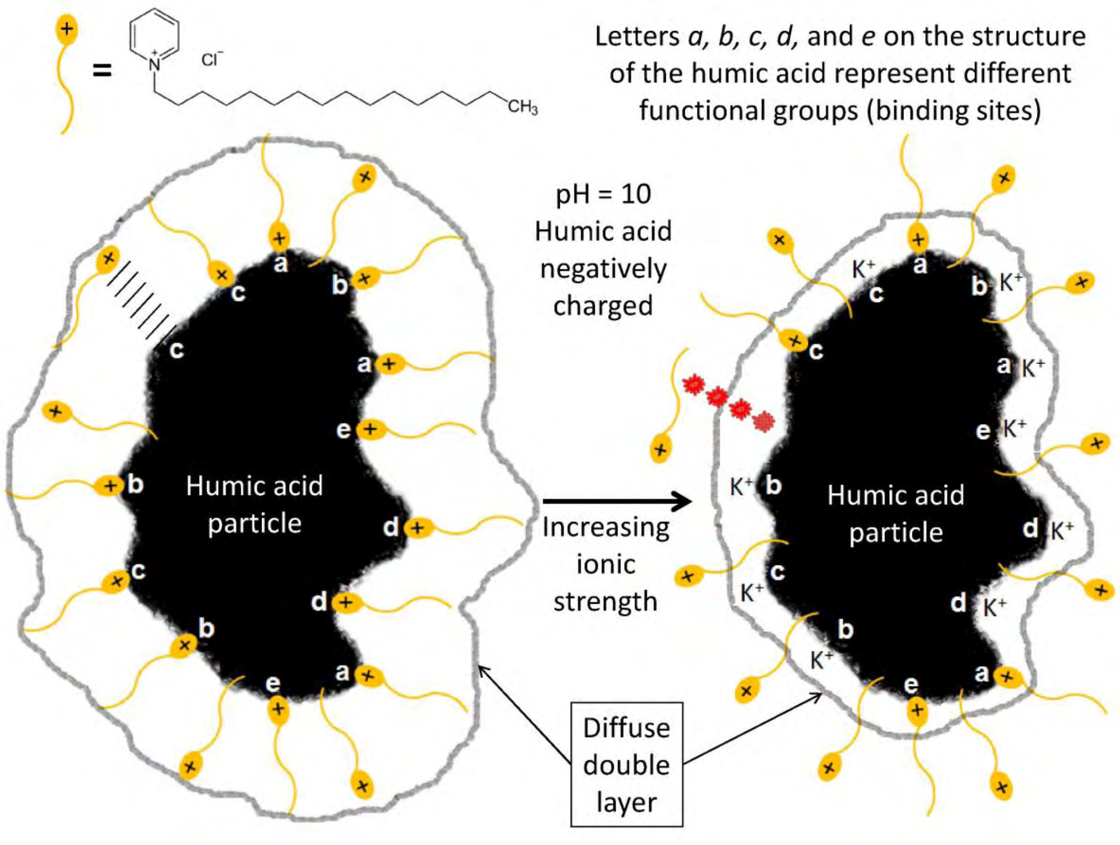
TOC art



ABSTRACT

Humic acids (HA) are a major representative of humic substances (HS), which are found in nearly all soils, sediments, and natural waters. They play a key role in many, if not most, chemical and physical properties in their environment. Despite of their importance, high complexity of HA makes them a poorly understood system. Therefore, understanding HA's physicochemical properties and interactions is crucial to understand their fundamental behavior and to obtain structural details. Cationic surfactants are known to interact electrostatic and hydrophobically with HA. Because they are a very well known and characterized system they represent a good choice of molecular probe to study HA. The objective of this work was to evaluate thermodynamically the binding between cationic surfactants and HA through isothermal titration calorimetry, aiming to get information about HA basic structure, the nature of this interaction, and if HA from different origins show different basic structures. Contrary to a putative supramolecular model, our evidence shows that HA structure is not loosely held. It did not show any division or conformational change when undergoing the interaction with surfactant. We also showed here that the basic structure of HA is kept virtually the same regarding differences on its sources and composition.

Keywords: Humic substances, isothermal titration calorimetry, microcalorimetry, interaction, and surfactants.

INTRODUCTION

Humic substances (HS) are found in nearly all soils, sediments, and natural waters. They are formed by chemical, physical, and biological transformation of plant, animal, and microorganisms matter into relatively stable and polydisperse particles. HS play a key role in many, if not most, chemical and physical properties in their environment. They are operationally fractionated into three groups as a function of their solubility at different pH. Fulvic acids (FA) are soluble at any pH, humic acids (HA) are soluble at pH > 2 and humins (HU) are entirely insoluble in aqueous solution [1]. HS have molecular weights varying from hundreds to thousands of Daltons and are known to be amphiphile molecules, with both hydrophilic and hydrophobic moieties in their structure. HS behave like a weak-acid polyelectrolyte and exhibit the ability to associate with different molecules by electrostatic and/or hydrophobic interactions [2, 3]. Also, as in most soils HS makes up the bulk of soil organic matter (SOM), they are very important to the global C cycle, once there is twice more carbon stabilized as SOM than there is in the whole atmosphere [4]. Still according to Amundson [4], the average C atom in atmospheric CO_2 passes through SOM somewhere in the world every ~12 years.

In spite of their importance, high heterogeneity and complexity of HS make them a poorly known and understood system [1, 5-7]. Therefore, understanding HS physicochemical properties and interactions is crucial to understand their fundamental behavior and to obtain structural details [8]. Similar to HS, surfactants are amphiphile molecules. Surfactants' structure makes it particularly favorable for them to reside at interfaces, that is why they are also termed surface active agents. Due to their amphiphilic nature and opposite charges, HS and cationic surfactants easily associate through both electrostatic and hydrofobic interactions [2, 3]. Therefore, surfactant molecules typify an ideal system to be used as a molecular probe to interact with HS and study such a complex system. One way to perform the thermodynamic study of this interaction is through isothermal titration calorimetry (ITC) technique.

ITC is a technique that combines thermochemical and analytical applications and directly measures the enthalpy change of any molecular interaction as a function of the amount of added reactant. It allows to monitor the binding process, determining binding isotherms and also giving, in some cases, direct information on the structure of the complex [9, 10]. The thermodynamic functions determined by ITC (enthalpy change - ΔH; entropy change - ΔS; free Gibbs energy change - ΔG; and calorific capacity - C_p) are useful for understanding the energetics behind any polymer-reactant interaction. The ITC technique is versatile, extremely sensitive and non selective regarding to polymers and reactants. The only requirement is that an enthalpy change is generated during the binding process. Due to its high sensitivity, ITC technique is also known as microcalorimetry. While the standard enthalpy of formation of one mol of water (~18 mL) is -285,800 J mol^{-1}, the actual calorimeters can sense heat changes as little as 0.000000001 J (nJ).

HA is a major component of HS and considered to be one of the most recalcitrant fractions in soil [11], thus it is a good representative of HS. We believe that, understanding the driving forces associated with HA-surfactant interaction will allow inferring about HA structure, organization, and behavior in the environment. According to the enthalpy changes involved in this interaction it will be possible to determine the electrostatic and/or hydrophobic character of this interaction, HA charge density, numbers of layers of surfactant binding onto HA, and if HA structure goes through any dissociation or conformational changes during its interaction with surfactants. The objective of this study was to evaluate thermodynamically the binding between cationic surfactants and HA under different ionic strengths, aiming to get the following information: (i) HA basic structure, (ii) the nature of this interaction, and (iii) if HA from different origins show different basic structures.

EXPERIMENTAL SECTION

Materials. Elliott Soil humic acid (ESHA), Suwannee River humic acid (SRHA), and Pahokee Peat humic acid (PPHA) were purchased from the International Humic Substances Society (IHSS). Cationic surfactant hexadecylpyridinium chloride monohydrate (CPC) was purchased from Sigma-Aldrich. The critical micelle concentration (cmc) value for CPC was 0.11 ± 0.03 mmol L^{-1} [3]. Humic acid (HA) solutions (0.1 mg mL^{-1}) and surfactant solutions (10.0 mmol L^{-1}) were prepared in a carbonate buffer pH 10 (6.4 mmol L^{-1} Na_2CO_3 + 6.0 mmol L^{-1} $NaHCO_3$) [3]. HA-surfactant interactions were evaluated in four ionic strengths (IS) (0.00, 0.05, 0.10, and 0.30 mol L^{-1}) adjusted with KCl salt.

Isothermal Titration Calorimetry (ITC). The enthalpy changes of HA-surfactant interactions in three different ionic strengths were performed in triplicate using a CSC-4200 microcalorimeter (Calorimeter Science Corp.) controlled by ItcRun software with a 1.75 mL reaction cell (sample and reference). The whole calorimetric procedure was chemically and electrically calibrated to the heat of protonation of (tris(hydroxymethyl)aminomethane) and the joule effect, as recommended [12]. Deionized water was used for preparing all solutions. The titrations were carried out through step-by-step injections (5 μL) of the concentrated surfactant solutions (titrant) with a gastight Hamilton syringe (250 μL), controlled by the calorimeter, with intervals of 60 min between each injection. These aliquots of concentrated surfactant solution were injected to the sample cell, under stirring at 300 rpm, and measurements were carried out at a constant temperature of 25.000 ±0.001 ^{o}C.

RESULTS AND DISCUSSION

An example of the titration curves obtained in this work is shown in figure 1, where the observed enthalpy changes (ΔH_{obs}) for each injection are plotted against the total surfactant concentration in the sample cell. In a typical experiment, there was an addition of 5 μL of surfactant aqueous solution (CPC 10 mmol L^{-1}) to (i) HA solution (0.1 mg mL^{-1}) and to (ii) pure carbonate buffer solution. From the titration of surfactant on pure buffer solution the following processes produce measurable enthalpy changes: (a) demicellization (ΔH_{demic}) – surfactant is titrated in micellar form, thus following the initial titrations these surfactant micelles dissociate into monomers, (b) the new interactions surfactant monomers are obligated to do once titrated in the solution (dilution; ΔH_{dil}), and (c) micellization (ΔH_{mic}) – when the surfactant concentration reaches the critical micelle concentration (*cmc)* again (0.11 mmol L^{-1}). This process can be summarized by the following equation:

$$\Delta H_{obs} = \Delta H_{demic} + \Delta H_{dil} + \Delta H_{mic} \qquad \text{Eq. 1}$$

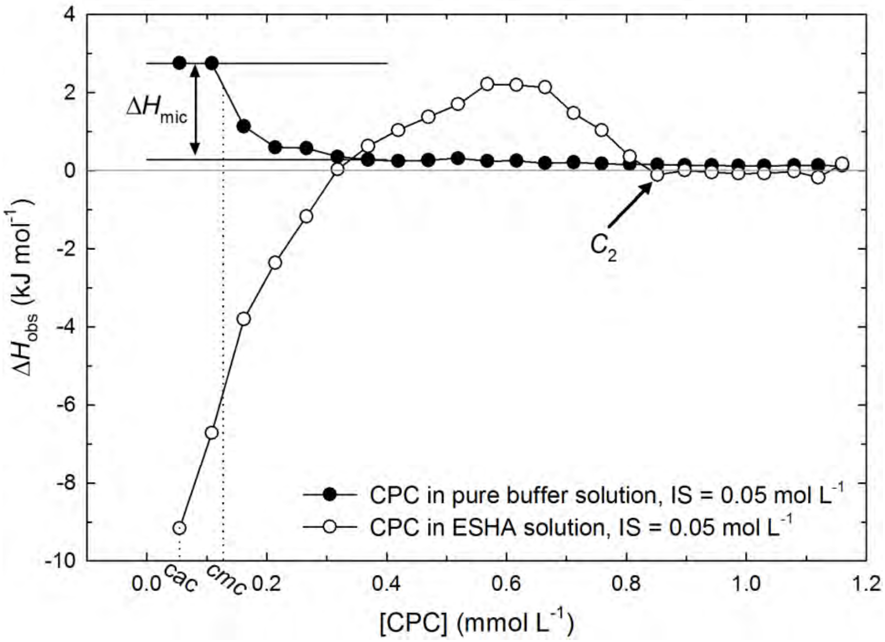


Figure 1. Calorimetric titration curves (observed enthalpy changes – ΔH_{obs}) from the addition of CPC surfactant solution (10.0 mmol L^{-1}) to Elliott soil humic acid (ESHA) solution (0.1 mg mL^{-1}) and its dilution in carbonate buffer at 25 ^{o}C and 0.05 mol L^{-1} ionic strength (IS). The acronyms cac, *cmc*, and C_2 stand for critical aggregation concentration, critical micelle concentration, and saturation concentration, respectively.

The difference in the enthalpy change between the two horizontal parts of the Ƨ-shaped curve, as marked on figure 1, is equal to ΔH_{mic} (-2.5 kJ mol^{-1}). From the titration of surfactant on HA solution (ΔH_{obs}), besides the processes cited above, there is an additional one that produces measurable enthalpy change – the interaction of surfactant with HA (ΔH_{int}).

$$\Delta H_{\mathrm{obs}} = \Delta H_{\mathrm{demic}} + \Delta H_{\mathrm{dil}} + \Delta H_{\mathrm{mic}} + \Delta H_{\mathrm{int}} \qquad \text{Eq. 2}$$

As the only difference between the curves on figure 1 is the presence of HA, CPC clearly interacts with HA. This HA-CPC interaction has also been shown by potentiometric measurements [3].

At low surfactant concentrations (or small surfactant-HA ratios, $R_{s/ha}$), individual surfactant molecules adsorb along the HA, which is characterized by a critical aggregation concentration (cac). At intermediate $R_{s/ha}$ values, a new process makes ΔH_{obs} become less endothermic, suggesting that surfactant monomers aggregate close to the HA structure. After HA saturation (saturation concentration, C_2), further addition of surfactant (increase of $R_{s/ha}$) promotes micelle formation in bulk solution [13]. Previous studies, using other surfactant molecules interaction systems, corroborate the molecular processes described above, including surface tension measurements [14], conductivity [15], dialysis [16], viscosity [17], dye solubilization [18], microcalorimetry [19], and scattering techniques [20]. The topic has also been treated in several very good review articles [10] and book chapters [21]. Usually, the beginning of the curves (titration on pure solution and on HA containing solution) is the same, and the point where they start to differentiate (cac) is reached after a couple of injections [22]. But since here both curves (in HA solution and in pure buffer solution; Figure 1) are different at the very beginning of the titration, we choose attribute cac to the CPC concentration reached after the first injection (0.055 mmol L^{-1}). In doing so we are following the protocols for cac determination (the point where curves start to differentiate), but we shall advise that the real cac is probably much smaller than the assigned one.

The first part of the curve of HA-surfactant interaction shows an exothermic pattern and that the heat released is decreasing at each injection. It goes on until exothermic heat ceases (0.32 mmol L^{-1} of CPC) and the curve acquires an endothermic pattern. This endothermic heat reaches its maximum and then starts to decrease until the point where both curves cross again and remain similar. The intersection of both curves indicates the point where HA is saturated with surfactant monomers (C_2 = 0.81 mmol L^{-1}) and free surfactant micelles start to form in the HA solution. At this point there is no more HA-surfactant interaction and that is the reason why curves overlap. In comparison, Barbosa *et al.* [22] studying PEO-SDS interaction found cac equal to 3.6 mmol L^{-1} of SDS for their system, and that PEO was saturated by SDS at a total concentration of 17.5 mmol L^{-1}. This result shows that the presence of charge on the HA particles makes HA-surfactant interaction stronger than PEO-SDS interaction, demonstrating a significant contribution of electrostatic energy for the HA-surfactant interaction.

In order to isolate the enthalpy changes related to HA-surfactant interactions only (Figure 2), we must subtract the titration curve in HA solution (ΔH_{obs}, Eq. 2) from the dilution curve in pure buffer solution (ΔH_{mic}, Eq. 1) at each surfactant concentration (curves showed in figure 1), according to the equation shown below:

$$\Delta H_{obs} - \Delta H_{mic} = \Delta H_{ap\text{-}int} \qquad \text{Eq. 3}$$

This way it is possible to evaluate the differential apparent enthalpy change for the HA-surfactant interactions ($\Delta H_{ap\text{-}int}$, Figure 2). Unfortunately, as the extent of binding (amount of surfactant adsorbed) is not known, we cannot calculate the exact molar enthalpy change of interaction, but only the apparent molar enthalpy change

($\Delta H_{\text{ap-int}}$). However, the features of the $\Delta H_{\text{ap-int}}$ curve give qualitative information about the progress of aggregation with increasing surfactant concentration [22].

By using ITC to investigate HA-surfactant interactions, it is possible to obtain four important parameters that characterize these interactions, namely (i) the critical aggregation concentration value (cac), (ii) the saturation concentration (C_2), (iii) the integral enthalpy change for aggregate formation (ΔH_{agg} (int)), and (iv) the number of mols of bounded surfactant per HA mass. In absence of isothermal binding data of CPC to HA we can calculate the integral enthalpy change for aggregate formation, ΔH_{agg}(int). It expresses the enthalpy change of the aggregation of one mol of surfactant onto HA, from the first injection up to C_2. Following Olofsson and Loh [23] for ΔH_{agg}(int) calculation, we assumed that C_2 had been reached after Y injections of concentrated surfactant solution (each injection added n^{inj} mols of surfactant) to give a total volume of V_Y. At C_2, we added a total surfactant equal to Yn^{inj}, but from this total, $V_Y[cmc]$ mols do not interact with HA. Naturally, the total energy measured, Σq_{obs}, should be discounted by the energy of demicellization and dilution, $Yq_{\text{demic+dil}}$. Mathematically, ΔH_{agg}(int) is calculated as follow and a summary of all these HA-surfactant parameters is presented in Table 1.

$$\Delta H_{\text{agg}}(\text{int}) = \left(\frac{\sum q_{obs} - Yq_{demic+dil}}{Yn^{inj} - V_Y[cmc]}\right) \qquad \text{Eq. 4}$$

From figure 2 it is possible to see that cationic surfactants strongly bind to HA structure, especially at pH 10, which is well above HA point of zero charge (pH 2-2.5). At the first injection -13.2 kJ mol^{-1} of heat were released from the HA-surfactant interaction. At the second injection the heat released was -12.9 kJ mol^{-1}, lower than the first one. The fact that the heat released is decreasing give valuable information about HA structure. Because at each injection the heat released is lower than the previous one, it shows that the surfactant monomers injected each time are binding to different sites at HA structure and that this binding is non-cooperative (ΔH does not change abruptly). Probably the different binding sites that surfactants are encountering at each concentration are due to the many different functional groups found on humic substances. If, instead of the pattern seen on figure 2 a straight line parallel to "x" axis was found, it would mean that surfactants were encountering the same type of binding sites each time.

Table 1. Thermodynamic parameters for humic acid-surfactant interactions at different ionic strengths.

HA type	IS (mol L^{-1})	*cmc* (mmol L^{-1})	cac (mmol L^{-1})	C_2 (mmol L^{-1})	ΔH_{agg}(int) (kJ mol^{-1})	Extent of binding of CPC on HA (mmol g^{-1})
SRHA	0.00	0.1	0.055	1.25	-8.65	13.78
PPHA	0.00	0.1	0.055	1.16	-9.02	12.65
ESHA	0.00	0.1	0.055	1.20	-8.76	13.22
PPHA	0.05	0.1	0.055	1.25	-3.64	13.78
ESHA	0.05	0.1	0.055	0.81	-3.74	8.10
PPHA	0.10	0.1	0.055	1.20	-1.52	13.22
ESHA	0.10	0.1	0.055	0.85	-1.84	8.67
ESHA	0.30	0.1	0.055	0.85	-0.19	8.67

PPHA – Pahokee Peat humic acid; ESHA – Elliott Soil humic acid; SRHA – Suwannee River humic acid; IS – ionic strength; *cmc* – critical micelle concentration; cac – critical aggregation concentration; C_2 – HA saturation concentration; ΔH_{agg}(int) – integral enthalpy change due to the aggregation.

As injections proceed, the heat released keeps decreasing until a point where the curve ceases to be exothermic and acquires an endothermic pattern, reaching a maximum $\Delta H_{ap\text{-}int}$ of 1.7 kJ mol^{-1} (Figure 2). At this point it is assumed that all the negatively charged sites on HA structure are bonded and that HA-surfactant interaction is now mainly hydrofobically driven, until the point where HA saturates (C_2 = 1.16

mmol L^{-1}) and no more enthalpy change is measured on the titration. The change from exothermic to endothermic pattern of the curve can be understood according to the following analysis:

$$\Delta H_{\text{ap-int}} = \Delta H_{\text{surf-sol}} + \Delta H_{\text{HA-sol}} + \Delta H_{\text{HA-surf}} \qquad \text{Eq. 5}$$

$$\left|\Delta H_{\text{surf-sol}} + \Delta H_{\text{HA-sol}}\right| < \left|\Delta H_{\text{HA-surf}}\right| \qquad \text{Eq. 6}$$

$$\left|\Delta H_{\text{surf-sol}} + \Delta H_{\text{HA-sol}}\right| > \left|\Delta H_{\text{HA-surf}}\right| \qquad \text{Eq. 7}$$

where $\Delta H_{\text{ap-int}}$ is the apparent interaction enthalpy change, $\Delta H_{\text{surf-sol}}$ is the enthalpy change due to the interactions between surfactant and solvent molecules, $\Delta H_{\text{HA-sol}}$ is the enthalpy change due to the interactions between HA and solvent molecules, and $\Delta H_{\text{HA-surf}}$ is the enthalpy change due to the interactions between HA and surfactant molecules.

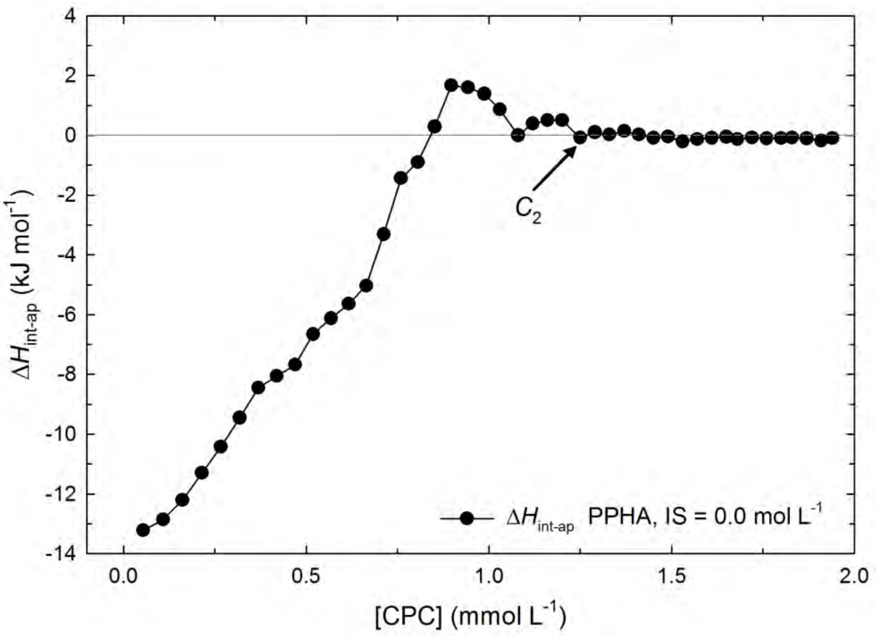


Figure 2. Apparent molar enthalpy change ($\Delta H_{\text{ap-int}}$) of interaction between Pahokee peat humic acid (PPHA) and CPC surfactant in carbonate buffer at 25 °C and 0.0 mol L^{-1} ionic strength (IS).

The first two components of Eq. 5 ($\Delta H_{\text{surf-sol}}$ and $\Delta H_{\text{HA-sol}}$) are due to the desolvation of both surfactant and HA and they are always endothermic, while the latter

($\Delta H_{\text{HA-surf}}$) is due to the HA-surfactant interaction and contributes negatively to the enthalpy change (exothermic). This $\Delta H_{\text{HA-surf}}$ that occurs here can happen due to electrostatic, covalent, dipole-dipole, ion-dipole, hydrogen bond, van der Waals, and hydrophobic interactions. Because the first part of the curve has shown an exothermic character (Figure 2), the exothermic component ($\Delta H_{\text{HA-surf}}$) of Eq. 5 must overcome the other two endothermic components (e.g. Eq. 6). We have attributed this strong HA-surfactant interaction to an electrostatic interaction between the negatively charged groups on the HA and the positively charged heads of the surfactants. When the curve starts to display the endothermic pattern, it means that the exothermic component ($\Delta H_{\text{HA-surf}}$) is not too strong anymore and the other two overcame it (e.g. Eq. 7). At this point we assume that HA still interacts with surfactant, but now the interaction is mainly hydrophobically driven. Hydrophobic force is a type of entropic force that originates from the entropy of the network of water molecules (interaction among them), thus it does not cause great enthalpy changes. Those favorable entropy changes due to hydrophobic interactions happen due to an increase in solvent entropy from burial of hydrophobic groups and release of water upon binding, as well as minimal loss of conformational degrees of freedom.

The amphiphilic nature of humic substances and their effects on water surface tension are well established. For this reason, humic substances were thought to be structured as an aggregation of smaller molecules in a micelle-like structure. Wershaw (1993) was one of the earliest to propose the “Membrane-micelle humus model” [24] and Piccolo *et al.* (1996) claimed to be the first showing direct evidence of the micelle-like or aggregate model of humic substances [25]. After that, new studies kept supporting the idea of the supramolecular model of humic substances, which depicts them as a self-assembling association of relatively small heterogeneous molecules held together by weak forces [5, 26, 27]. The most accepted model nowadays still keeps the supramolecular idea. Based on NMR spectroscopy studies, it has been shown that the supramolecular structure is made of known biochemical compounds such as lignin fragments, alcohols, aliphatic acids, ethers, esters, polysaccharides, polypeptides, and many others, and that metal cations play an important role on its complexation [28]. These authors also pointed that this supramolecular structure varies with material origin and extraction method.

Even though this supramolecular arrangement is the more likely way humic substances behave on the environment, it does not represent the basic structure of a single HA molecule. At least not so weakly bonded that will allow them to separate at the simple presence of organic acids [29], for example. According to our results, the basic HA structure, or HA monomers, shows a dense compact structure that does not go through any division or conformational change when undergoing an interaction. It is so because $\Delta H_{\text{ap-int}}$ *vs* [CPC] curve is monotone, without any change on concavity (Figure 2). If HA structure was undergoing any division or conformational changes, as it is believed that they would, it would be seen peaks and valleys as features of the curve [30]. It would show great ΔH changes due to changes in entropy happening due to the new parts of the HA structure exposed to the solution, and exothermic heats due to the aggregation of surfactants to new adsorption sites. As mostly of those techniques used to build this putative supramolecular model require solutions with relatively high HA concentration, what they named as HA supramolecular structure could be just an aggregation (micelle-like) of the truly basic structure of HA, as already evidenced by Lam and Simpson (2009) [31] and Smejkalova and Piccolo (2008) [32]. They showed that as HA concentration increases, a noticeable shift in molecular size distribution occurs, from smaller to larger components, indicating a self-association of humic molecules. Therefore, the relatively high HS concentrations required for chromatographic and spectroscopic experiments would explain much of all the variation regarding to humic substances' structure, size, and behavior found so far.

The decomposition process that leads to the formation of humic substances differs according to environmental characteristics. There is a great importance of biotic factors on this process in tropical ecosystems, while unique factors affect decomposition in arid and semi-arid ecosystems, including abiotic controls such as photodegradation [33]. It is widespread in the literature that humic substances from different origins and/or that went through different formation processes are qualitatively different from each other [34]. The proportion of humic substances' components is believed to be related to source, maturity, depositional environment and degree of degradation [35]. Although a number of properties are common to various aquatic and terrestrial humic substances, some authors can distinguish their nature and origin by elemental and spectroscopic analysis [36]. Studying humic substances isolated from swamp water, soil, peat and brown coal by means of elemental composition, ^{13}C NMR, and Py-GC/MS, Lu et al. (2000) [35] concluded that they show similar chemical composition but different structures. In order to determine how different, in terms of molecular surfactant probe, the basic structure of different HA could be, we choose HA from three distinct sources (soil, peat and river) and proceeded the microcalorimetric study of the HA-surfactant interaction for each one of them (Figure 3). According to the International Humic Substances Society, the chemical properties of these three HA are fairly different in terms of elemental composition, stable isotopic ratios, amino acid composition, carbohydrate composition, and functional groups. From the features of the curves of these three humic acids (Figure 3) it is possible to notice that, despite the differences regarding their origin or formation, they share a common basic structure. Perhaps PPHA has more hydrophobic sites than ESHA and SRHA due to the higher endothermic peak, but still the similarity of the curves shows that they share a common basic structure (Figure 3). In agreement with that, they also showed very similar C_2, ΔH_{agg}(int), and the extent of binding of CPC at 0.00 mol L^{-1} IS (Table 1).

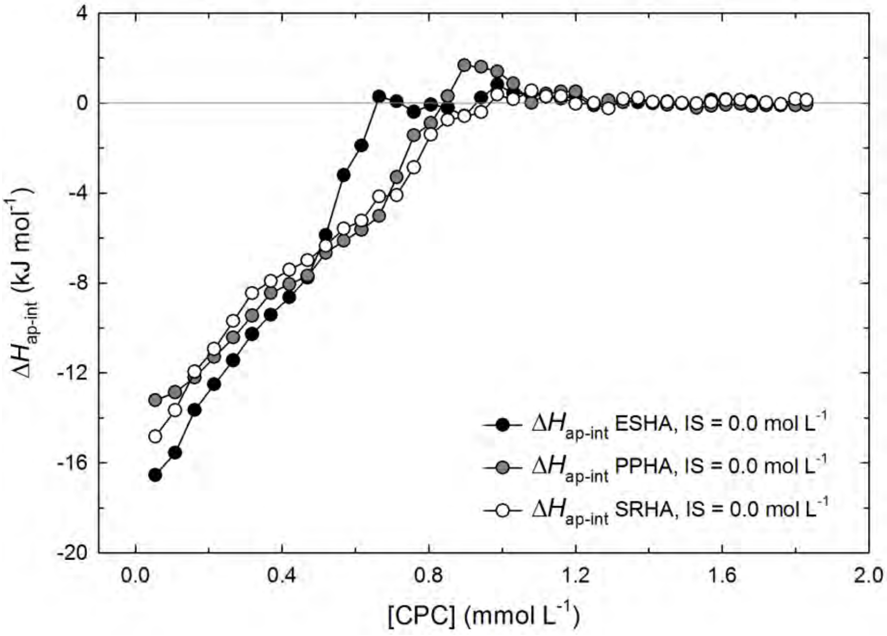


Figure 3. Apparent molar enthalpy change ($\Delta H_{ap\text{-}int}$) of the interaction between Elliott soil, Pahokee peat, and Suwannee river humic acids (ESHA, PPHA, and SRHA) and CPC surfactant in carbonate buffer at 25 °C and 0.0 mol L^{-1} ionic strength (IS).

But even though figure 3 shows that different HA share a common basic structure, it also shows that the binding sites are different among them, once the heat released is not the same at each surfactant injection for all three HA. Instead, if the injection points were overlapping each other, it would indicate that there was no difference among the binding sites for the different HAs. The binding of those three HA from different sources with CPC showed a strong interaction starting from very small amounts of surfactants injected. For the first injection of surfactant on HA solutions, HA-surfactant interaction released -16.6, -14.8, and -13.2 kJ mol^{-1} for soil (ESHA), river (SRHA), and peat (PPHA) HA, respectively. As surfactant concentration increased, the different heat released kept showing differences on the binding sites.

These differences on the heat released at each injection allow us to differentiate HAs through ITC technique and are probably due to the great variety of functional groups present on those HA's structure. It worth noting that, based on the features of the titration curves, the interaction of surfactants to the HA structure occurs as a monolayer. If the interaction were occurring in more than a single layer, it would be seen as many parallel vertical lines as surfactant layers were adsorbing onto each HA.

As already mentioned, a variety of intermolecular interactions could be the source of the enthalpy change due to the HA-surfactant interaction ($\Delta H_{\text{HA-surf}}$). In order to evaluate the weight of electrostatic interaction on $\Delta H_{\text{HA-surf}}$ we proceed the same interaction studies in solutions with increasingly ionic strength (IS). Negatively charged HAs in solution will attract cations while repel anions. In this way, a concentrated layer of cations is formed around them. Assuming that these cations do not adsorb onto the surface, they are denoted counterions and constitute the diffuse double layer (DDL). The thickness of this DDL is such that this layer contains a sufficient number of counterions to neutralize the surface charge in order to maintain the system electric neutrality. The electrical potential at the surface of shear is defined as the zeta potential (ζ), and this is the value that is typically used to characterize the electrical properties of the surface. Thus, as IS (ions bulk concentration) increases, the thickness of DDL decreases because ζ decreases and less volume is required to contain enough counterions to neutralize the surface charge. In other words, increasing IS shields the negatively charges of HA in solution. So, once the increase of IS leads to a decrease of ζ, if the nature of HA-surfactant interaction is electrostatic, the increase on solution's IS would cause the heat released from this interaction to decrease, allowing us to pinpoint the main type of interaction that is occurring.

As it is shown on figure 4, increasing the IS of the solution leads to a drastic reduction on the heat released from the HA-surfactant interaction, confirming that the binding of surfactants to HA have an important contribution from electrostatic interaction. The $\Delta H_{\text{ap-int}}$ for the binding of surfactant onto ESHA at the first injection was -16.5, -9.2, -6.4, and -2.8 kJ mol^{-1} for 0.00, 0.05, 0.10, and 0.30 mol L^{-1} IS. For PPHA these values were -13.2, -9.0, and -7.2 kJ mol^{-1} for 0.00, 0.05, and 0.10 mol L^{-1} IS. Even though HA's amphiphile structure allow them to interact both hydrophobic and electrostatically on the environment, at low ionic strengths (IS = 0) HA's hydrophobicity does not play an important role and the interaction is basically electrostatically driven (Figure 4). But when facing an increasingly IS environment, hydrophobic interaction gets more important, as it can be seen by the increase on the endothermic part of the curves (Figure 4). At higher IS, hydrophobic forces play an important role on HA-surfactant interaction. It also can be seen by the decrease of ΔH_{agg}(int) with the increase on IS (Table 1). The ΔH_{agg}(int) of the HA-surfactant interaction of ESHA went from -8.76 kJ mol^{-1} at 0.00 mol L^{-1} IS to -0.19 kJ mol^{-1} at 0.30 mol L^{-1} IS. This difference confirms the change from electrostatic nature of the interaction at low IS to hydrophobic nature at high IS. But even though hydrophobic forces play big role on the interaction at high IS, the negative ΔH_{agg}(int) of -0.19 kJ mol^{-1} tells us that the interaction as a hole still show an exothermic pattern.

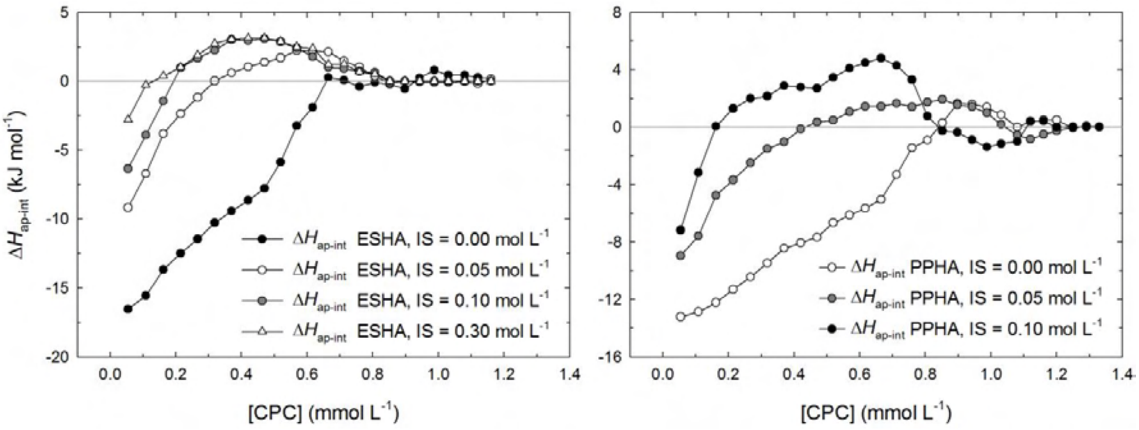


Figure 4. Apparent molar enthalpy change ($\Delta H_{ap\text{-}int}$) of the interaction between Elliott soil and Pahokee peat humic acids (ESHA and PPHA) and CPC surfactant in carbonate buffer (25 ^{o}C) under increasingly ionic strength (IS).

Another interesting thing to notice from these results is the change on C_2 and on the binding extent with increasing IS (Table 1). While C_2 and the binding extent do not change for PPHA with the increase on IS, they do change for ESHA, getting smaller with the increase of IS. It means that for ESHA the increase on IS is not just shielding the HA charged sites, changing the nature of the interaction, but it is also diminishing the extent of the interaction. So, the use of solutions with different IS also showed to be useful on differentiating HA. Besides the changes on C_2 and binding extent, changes on the heat involved on the titration of each HA behave differently with the increase of IS. For ESHA the heat released at the first injection decreased 44.2 and 61.2% when IS was increased to 0.05 and 0.10 mol L^{-1}, respectively, while for PPHA, the heat released decreased 31.8 and 45.4% after the IS was increased to 0.05 and 0.10 mol L^{-1}, respectively.

In summary, we showed here a new approach to study humic substances using surfactants as molecular probes through ITC technique (microcalorimetry). To our knowledge it is the first time it has been done and it has shown promising results. The heat pattern of the interaction between HA and surfactant showed that, in fact, there is a dense unchanging and indivisible basic structure of HA, contrary to what the so called supramolecular model preaches. If the supramolecular model indeed represents HS's structure, they are not loosely held together as widespread in the literature. Instead, what researchers are seeing perhaps is the aggregation of the truly basic structure of HS, due to the necessity of high HS concentration solutions in their experiments. We also showed here that this basic structure of HA is rather similar in the environment, being virtually the same for HA of different sources with different compositions (soil, peat, and river sources). Finally, we showed that HA-surfactant interaction is mainly electrostatic, at least at low IS (IS = 0). As IS increases the interaction pattern shifts from electrostatic to hydrophobic. This work brings new information on HA's interaction and structure by employing a relatively recent technique, especially regarding to this field. To the best of our knowledge we are the first to show evidence of the existence of HA monomers. It improves the knowledge about such complex system and brings a new tool to study humic substances.

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CHAPTER 3

Likely output journal *Agriculture, Ecosystems & Environment*

THE ROLE OF SOIL TEXTURE AND CLIMATIC CONDITIONS ONTO SOIL ORGANIC CARBON IN THREE MAJOR BRAZILIAN BIOMES

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HIGHLIGHTS

- There is room to increase C sequestration deeper in Brazilian clayey soils.
- Increasing temperature is positively correlated to an increase on SOC mineralization after land use change.
- Higher SOC stocks are associated with higher C/N.
- Pasture plantation is leading to negative changes on SOC, while eucalyptus is not.
- Biomes differed in relation to their SOC vertical distribution.

ABSTRACT

Climate changes caused by increased atmospheric concentration of CO_2 have stimulated studies seeking a better understanding of the global C cycle. Soils are a very dynamic compartment of this cycle with higher C content than plant biomass and atmosphere. Since the global role of soil organic C (SOC) and its sensitivity to climatic conditions are still not well understood, and to improve overall SOC estimates globally, country level studies of SOC are needed. The objectives of this work were to quantify SOC in soils under native vegetation and short-rotation eucalypt and pasture plantations in three major Brazilian biomes, and evaluate the associations of SOC with climatic conditions and clay content. SOC stocks at the first soil meter under native vegetation, eucalypt, and pasture averaged 137.3, 127.9, and 127.1 t ha^{-1}, respectively. The highest SOC stock found in this work was under eucalyptus cultivation in the Pampas, 177.6 t ha^{-1}. Our results showed that SOC correlated positively with soil clay content, but that this relationship is not linear towards high clay content soils, especially in deeper layers, suggesting that those clay particles are not saturated with SOC and that there is still room to improve C sequestration. Another important outcome of our work is that, even though SOC stocks did not correlate with temperature, SOC mineralization after land use change was positively correlated with it, making it more difficult to remediate soil C sequestration in a climate change scenario.

Keywords: Soil organic matter, free light organic matter, total organic carbon, Brazil, Cerrado, savannah, Atlantic Forest, Pampas, grasslands, C/N, eucalypt, pasture.

1. INTRODUCTION

Climate changes caused by increased atmospheric concentration of CO_2 have stimulated a number of studies seeking a better understanding of the global C cycle. Soils are one of the main compartments of this cycle with higher C content than plant biomass and atmosphere combined (Amundson, 2001). It is estimated that ~1502 gt of C are storaged as soil organic C (SOC) at the first meter of soils, value that increases to ~2344 gt if the three first meters are taking into account (Jobbágy and Jackson, 2000). Still according to these authors temperate forests and grasslands show an average of 160 and 117 t ha^{-1} of SOC in the first meter of soil, while tropical forests and grasslands/savannas show an average of 172 and 132 t ha^{-1}, and crop fields 112 t ha^{-1} of SOC in the first meter of soil.

Globally, soil C sequestration offsets C released by land use changes, with a positive balance of 1.1 gt of C sequestered per year (Normile, 2009a). But the increasingly need for land to produce food, wood, and many other raw materials have brought more attention to the SOC compartment due to its magnitude and dynamically characteristics. On average, each C atom in atmospheric CO_2 passes through soil organic matter (SOM) somewhere in the world every ~12 years (Amundson, 2001). This becomes especially important to Brazil once its emissions pattern is different from those of the developed countries. In Brazil, the majority of the greenhouse gases emissions come from land use change and agriculture.

The terrestrial vegetation sequester a quarter of all CO_2 released annually into the atmosphere (Normile, 2009b), with tropical forests and savannas accounting for 60% of it (Beer et al., 2010). Due to the importance of photosynthesis to CO_2 sequestration, planted forests can become an important agent for C sequestration due to its high productivity (Jackson et al., 2005). This becomes particularly important to Brazil, where the forestry industry is booming. Additionally, eucalyptus, the main forest species grown in the country, has the shortest rotation length among all tree species used to produce wood and cellulose pulp cultivated around the world (Sixta, 2006). However, for those forests actually contribute to the sequestration of atmospheric CO_2, its management should at least maintain the original soil C levels, ensuring that C sequestration promoted by trees is not neutralized by C losses due to the accelerated oxidation of SOM.

Many empirical relationships have been proposed to relate SOC stocks with environmental factors (Rodríguez-Murillo, 2001). In general, climatic features such as mean annual precipitation and mean annual temperature and soil texture determine regional patterns of SOC stocks (Jobbágy and Jackson, 2000). It happens because these factors influence the terrestrial gross primary production, organisms’ activity and stabilization and protection of SOM. Keeping other environmental factors constant, clay content has positive correlation with SOC stocks in most soils (Six et al., 2002; Stevenson, 1994; Stewart et al., 2008; Zinn et al., 2007). The importance of large specific surface area and charge density of clay size materials for any reaction in soils are widely known. It increases the adsorption of colloidal soluble organic substances (Zinn et al., 2007), provides chemical and physical protection to SOC and influence microbial activity, leading to an increase on the production of polymers looking like humic substances (resynthesis products) (Zech et al., 1997).

In order to improve overall estimates of SOC stocks globally, regional studies are needed, especially in countries like Brazil, where such data are still scarce (Bernoux et al., 2002). Also, the global role of soil C stocks and their sensitivity to climatic conditions are still not well understood (Gianelle et al., 2010), what becomes especially important in a global climate change scenario. With those issues in mind the objectives of this work were: 1) quantify total organic C (TOC) and C on free light organic matter (C_{FLOM}), and C/N of total SOC (C/N_{TOTAL}) and of free light organic matter (C/N_{FLOM}) fractions in soils of three major Brazilian biomes (Atlantic Forest, Cerrado – savannah , and Pampas – grassland) under native vegetation, eucalyptus, and pasture; 2) evaluate the changes on TOC (ΔTOC) and C_{FLOM} (ΔC_{FLOM}) and on C/N_{FLOM} ($\Delta C/N_{FLOM}$) and C/N_{TOTAL} ($\Delta C/N_{TOTAL}$) after land use changes (eucalyptus and pasture) comparing to native vegetation; 3) assess the influence of clay and climatic characteristics on TOC and C_{FLOM}, C/N_{TOTAL} and C/N_{FLOM}, ΔTOC and ΔC_{FLOM}, and $\Delta C/N_{TOTAL}$ and $\Delta C/N_{FLOM}$.

2. MATERIAL AND METHODS

The data used here came from studies conducted on areas where eucalyptus plantation is more expressive in Brazil. These areas are located within three major Brazilian biomes, namely, Atlantic Forest, Cerrado (savanna), and Pampas (grasslands).

In 2004, a soil sampling was performed in 15 places (five different states) located within these three biomes (APPENDIX). On each one of these places, soils under eucalyptus plantation and nearby reference area (native vegetation) were sampled. Additionally, when there was a pasture area adjacent to these eucalyptus/native vegetation pairs, it was also sampled. We sampled 17 areas of native vegetation (Cerrado = 9, Atlantic Forest = 7, and Pampas = 1), 47 eucalyptus plantations (Cerrado = 24, Atlantic Forest = 17, and Pampas = 6), and 10 pasture areas (Cerrado = 5 and Atlantic Forest = 5), a total of 57 sampling areas. These areas were sampled from 0-10, 10-20, 20-40, 40-60, and 60-100 cm. Core samples were taken for chemical analysis and for physical characterization purposes a trench was opened on each site and samples taken using volumetric rings. Samples for chemical characterization were taken in four replicates, with each one of these replicates being composed by four simple samples. Detailed information about all sites, as well as all chemical and physical characterization of the samples is supplied on the APPENDIX.

The determination of total organic C (TOC) was carried out by wet digestion (Yeomans and Bremner, 1988) and total N by Kjeldahl method. Free light organic matter (FLOM) was separated by density fractionation using a 1.8 g mL^{-1} NaI solution (Sohi et al., 2001) and its levels of C and N determined by dry combustion (elemental analyzer). To avoid overestimate TOC and C_{FLOM} stocks on eucalyptus and pasture areas due to soil compaction, soil bulk density of the reference areas (native vegetation) were used in the calculations. In order to calculate the changes in TOC, C_{FLOM}, C/N_{TOTAL}, and C/N_{FLOM} we used the following formula:

$$\Delta x = \left(\frac{x_{\mathrm{CULT}}}{x_{\mathrm{REF}}} - 1\right) 100 \qquad (1)$$

where x represents either TOC, C_{FLOM}, C/N_{TOTAL}, or C/N_{FLOM}; x_{CULT} is the given variable on the areas cultivated with eucalyptus or pasture; and x_{REF} is the same given variable on the reference area (native vegetation). This formula returns the proportional change (%) of a given variable after land use change (eucalyptus or pasture plantation) in relation to the reference area (native vegetation). A positive change translates to a proportional increase of the given variable on cultivated areas in comparison to the native vegetation. On the other hand, a negative change means that land use change is leading to a decrease of the studied variable.

Due to the unbalanced number of repetitions of our variables, in order to compare averages of SOM variables and Δs (TOC and C_{FLOM}, ΔTOC and ΔC_{FLOM}, C/N_{TOTAL} and C/N_{FLOM}, and $\Delta C/N_{TOTAL}$ and $\Delta C/N_{FLOM}$) we used the 95% confidence intervals. Differences between two variables as well as differences from 0% for Δs were assessed as non-overlapping 95% confidence intervals. To estimate relationships between SOM variables, and between them and clay content and climatic characteristics we used Pearson correlations. We also proceed linear and multiple stepwise regression analysis between the variables cited above and clay content and climatic characteristics. Throughout the text the symbols °, *, ** and *** are used and refer to significance levels of 10, 5, 1, and 0.1 %, respectively, and *ns* means non-significant at 10% of significance. The climatic characteristics used in this work were: mean maximum temperature (^oC, T_{max}), mean minimum temperature (^oC, T_{min}), mean temperature amplitude within a day (^oC, T_{amp}), average temperature (^oC, T_{ave}), mean annual precipitation (mm, *MAP*), mean monthly relative humidity (%, *RH*), mean annual vapor pressure (hpa, *VP*), total evapotranspiration within a year (mm, *ETP*), total water deficit within a year (mm, *DEF*), and annual water surplus (mm, *SURP*), which represents the water percolated below the root system.

3. RESULTS AND DISCUSSION

3.1 TOC and C_{FLOM} stocks and C/N_{TOTAL} and C/N_{FLOM} in three Brazilian biome's soils under native vegetation, eucalyptus plantation, and pasture

In general, the average TOC stock under native vegetation (including the three biomes) at the first meter was 137.3 t ha^{-1}, which was not significantly different ($P < 0.05$) from TOC stocks under eucalyptus and pasture plantations (127.9 and 127.1 t ha^{-1}, respectively; Figure 1). When looking at each biome separately, Cerrado biome showed the same behavior as for all data in general, where TOC stocks under native vegetation, eucalyptus, and pasture did not differ significantly ($P < 0.05$). On the other hand, eucalyptus plantation in the Atlantic Forest biome showed a significant lower TOC stock than under native vegetation, while in the Pampas eucalyptus plantations led to a significant increase on the TOC stock compared to native vegetation ($P < 0.05$). In fact, eucalyptus plantations in the Pampas region showed the highest TOC stock at the first soil meter found in this work (177.6 t ha^{-1}, Figure 1). These results are of practical importance for the eucalyptus cultivation in Brazil, once the majority of the areas are located in the Atlantic Forest biome. The TOC stocks that we showed here are in agreement with the global estimative by Jobbágy and Jackson (2000), which found TOC stocks for tropical sites varying from 132 to 186 t ha^{-1}, for grassland/savanna and evergreen forests, respectively, and around 112 t ha^{-1} for crops.

Differently from TOC stocks, C_{FLOM} stocks in eucalyptus and pasture areas generally were significant lower than the native vegetation (Figure 1; $P < 0.05$). This trend of lower C_{FLOM} stock under plantations held throughout all biomes, but the amount of C_{FLOM} stocks were significantly different among them. C_{FLOM} stocks were higher in the Cerrado biome, followed by the Atlantic Forest biome, and lastly by the Pampas (Figure 1). Regarding to the quality of SOM and FLOM, FLOM from pasture areas showed high C/N_{FLOM}, but this characteristic was not passed to the C/N_{TOTAL}, where pasture areas showed the lowest C/N_{TOTAL}. An interesting thing to notice here is that, even though C/N_{FLOM} was different among biomes, the humification process seems to smooth those differences, producing a SOM with similar qualities (C/N_{TOTAL}).

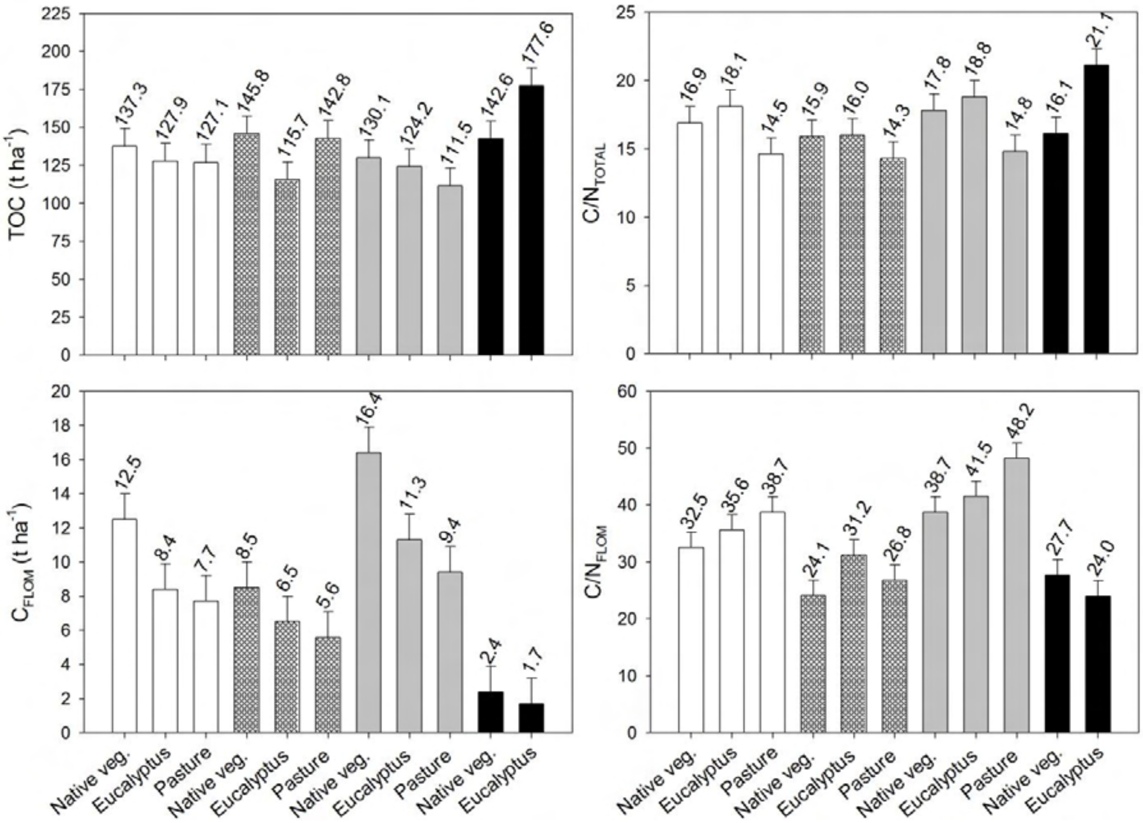

Figure 1. Mean values of total organic C (TOC) and C on free light organic matter (C_{FLOM}) stocks and C/N_{TOTAL} and C/N_{FLOM} for 0-100 cm depth. These mean values are given for native vegetation, eucalyptus and pasture areas, in general (white bars) and for each biome separately (dashed bars – Atlantic Forest, gray bars – Cerrado, and black bars – Pampas). The bars on the top of each column represent the 95% confidence interval.

Plant material's characteristics and microbial activity are known to be the main factors determining SOM C/N. The deposition of more labile compounds can induce SOC oxidation (priming effect) (Fontaine et al., 2007; Hamer and Marschner, 2005), leading to a decrease of soil C/N. On the other hand, substrates with a higher content of lignin and poliphenols are harder to metabolize, leading to an increase of this ratio. Small differences on the decomposition pace can lead to big differences in soil C/N over the time (Finzi et al., 1998). Generally, soil C/N tends to be closer to that one of the microbial community present in it (Janssen, 1996), once the microorganisms in the soil strive to maintain a healthy balance between nitrogen and carbon in their diet, pumping out more carbon as CO_2 when carbon rich materials are added (Manzoni et al., 2008). Our results corroborate to these findings once FLOM quality (C/N_{FLOM}) differed among biomes, while C/N_{TOTAL} was rather similar among them (Figure 1).

The set of edaphoclimatic characteristics that defines biomes influence differently the factors that control C soil cycle (Lemenih and Itanna, 2004). This becomes clear when looking at TOC and C_{FLOM} stocks (Figure 1). Although FLOM represents a SOM fraction sensitive to soil management, its relationship with TOC was shown to be different and variable among biomes. The lowest C stocks in FLOM found in the Pampas biome's soils led to the largest TOC stocks. In this biome, even with the reduced C_{FLOM} stocks under eucalyptus plantations, TOC stock was the highest. One of the causes of this pattern could be the cooler temperatures on the Pampas' area, which could affects microbial activity and lengthens SOM turnover, favoring the accumulation of SOC (Stevenson, 1994). Additionally, soils of this region are less weathered than the Cerrado and Atlantic Forest soils, with 1:1 and 2:1 clay minerals in its composition, which are more effective in protecting and stabilizing the SOC (Zech et al., 1997).

As already mentioned, TOC stocks at the first soil meter under native vegetation, eucalyptus, and pasture, in general, showed no differences (Figure 1; $P < 0.05$). But looking to the changes caused on SOM fractions and quality by eucalyptus and pasture cultivation, interesting things arise (Figure 2). In general, eucalyptus cultivation did not cause any significant change on TOC nor on C_{FLOM} stocks ($P < 0.05$). However, it did cause C/N_{TOTAL} and C/N_{FLOM} to increase. On the other hand, pasture cultivation also led to a positive change on C/N_{FLOM}, but it causes TOC and

C_{FLOM} to decrease significantly ($P < 0.05$). It means that the pasture areas sampled in this work are showing a negative impact on SOC, acting as a source of CO_2 to the atmosphere. It is known that soils under well managed pastures have high potential for accumulation and stabilization of C (Cerri et al., 2007), given the high input of plant material in those soils, specially underground (roots). But pastures in Brazil are usually a poorly managed agricultural system, with little input of technology. According to EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria), there are approximately 150 million hectares of pastures in Brazil that show any kind of problem, being degradation the most worrisome of them. This, added to the fact that Brazilian soils are generally acid and low in fertility leads to a system with low primary productivity, thus with low input of plant material to the soils, leading to a decrease on the original stocks of TOC and C_{FLOM}.

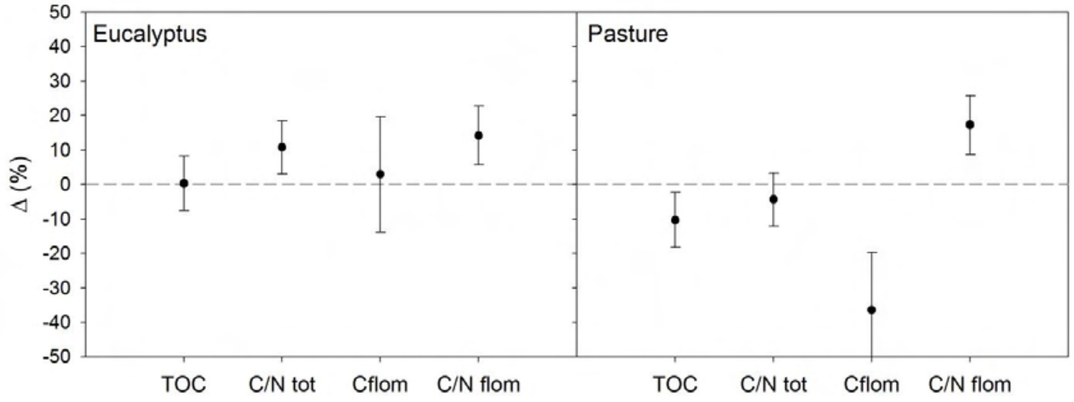

Figure 2. Average changes (Δ) on total organic C (TOC), C on free light organic matter (C_{FLOM}), C/N_{TOTAL}, and C/N_{FLOM} after land use change (eucalyptus or pasture plantation) compared to the reference area (native vegetation), for 0-100 cm depth.

Regarding the vertical distribution of SOC it was found to be different among biomes (Figure 3), but not clearly different among land uses (native vegetation, eucalyptus, and pasture). The relative distribution of SOC in the first meter of soil was deepest in Pampas and similarly shallower in Atlantic forest and Cerrado biomes. In the

Pampas region 46 % of all SOC in the first meter of soil was contained in the lower 40 cm (60 – 100 cm layer; Figure 3). These results are not in agreement with Jobbágy and Jackson (2000) whose data showed that SOC distributed more deeply as precipitation decreased and temperature increased. Of course they found this trend on a global database, which does not exclude the pattern seen here on a smaller country level. But we cannot deny that our data may be biased by the smaller subset of data from the Pampas. With a smaller subset we miss, for example, clay content and climatic characteristics variation on the samples from this biome.

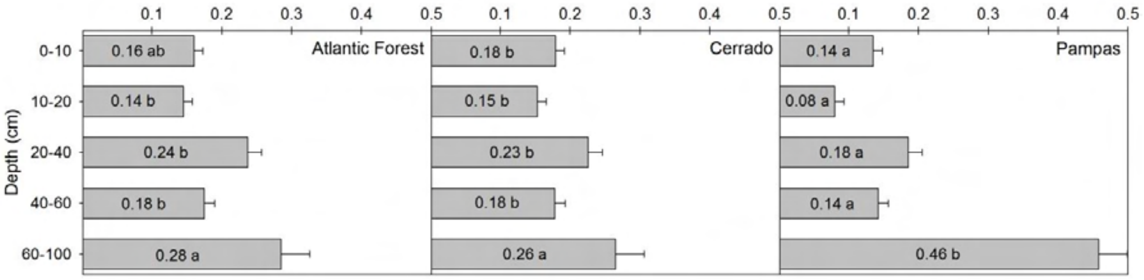

Figure 3. Proportional distribution of total organic C (TOC) in the first soil meter of each biome. Bars sum to one and letters indicate significant differences among biomes at each depth interval (non-overlapping 95% confidence interval, $P < 0.05$).

3.2 *Associations of TOC, ΔTOC, C_{FLOM}, ΔC_{FLOM}, C/N_{TOTAL}, $\Delta C/N_{TOTAL}$, C/N_{FLOM}, and $\Delta C/N_{FLOM}$ with climatic characteristics and clay content*

For the first meter of soil our results showed that, in general, TOC correlates positively with clay content but negatively with temperature, vapor pressure, and evapotranspiration (Table 1). C_{FLOM} also correlated positively with clay content, but to a lesser extent, and it correlated negatively with water availability indexes (negatively with MAP and SURP and positively with DEF). This lack of association of TOC with MAP disagrees with the fact that regional patterns of SOC are positively associated with MAP and clay in a diverse array of soils and vegetation types (Jobbágy and

Jackson, 2000; Stevenson, 1994). But according to the Rothamsted C model parameters, increasing soil moisture by 10% of pore space may multiply decay rates by a factor of 1.2 up to 1.8 (Jenkinson et al., 1992). It is because water availability constrains both plant production and decomposition (Austin and Vitousek, 1998; Pastor and Post, 1986).

Table 1. Correlation of soil organic matter fractions and characteristics with clay content and climatic characteristics for 0-100 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N$_{TOT}$	Δ C/N$_{TOT}$	C$_{FLOM}$ (g kg^{-1})	C$_{FLOM}$ (t ha^{-1})	Δ C$_{FLOM}$	C/N$_{FLOM}$	Δ C/N$_{FLOM}$
TOC t ha^{-1}	0.90***									
Δ TOC	0.34**	0.36**								
C/N$_{TOTAL}$	0.46***	0.46***	0.52***							
Δ C/N$_{TOTAL}$	0.42**	0.55***	0.37**	0.83***						
C$_{FLOM}$ g kg^{-1}	0.48***	*ns*	*ns*	0.28*	*ns*					
C$_{FLOM}$ t ha^{-1}	0.36**	*ns*	*ns*	*ns*	*ns*	0.94***				
Δ C$_{FLOM}$	*ns*	*ns*	0.70***	*ns*	*ns*	0.40**	0.41**			
C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	0.33**	0.36**	*ns*		
Δ C/N$_{FLOM}$	-0.43**	-0.35**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.61***	
Clay	0.67***	0.61***	*ns*	*ns*	*ns*	0.37**	0.34**	*ns*	0.36**	*ns*
MAP	*ns*	*ns*	*ns*	*ns*	*ns*	-0.49***	-0.50***	-0.33*	-0.49***	*ns*
Tave	-0.35**	-0.30**	-0.42**	-0.39**	*ns*	*ns*	0.25*	*ns*	0.26*	0.46***
Tmax	-0.33**	-0.27*	-0.44**	-0.27*	*ns*	*ns*	*ns*	*ns*	0.24*	0.46***
Tmin	-0.41***	-0.35**	-0.29*	-0.55***	-0.42**	*ns*	*ns*	*ns*	*ns*	0.35**
Tamp	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
VP	-0.41***	-0.42***	*ns*	-0.39**	-0.44**	*ns*	*ns*	*ns*	*ns*	*ns*
ETP	-0.40***	-0.33**	-0.38**	-0.55***	-0.38**	*ns*	*ns*	*ns*	*ns*	0.44**
DEF	*ns*	*ns*	*ns*	-0.24*	-0.37**	0.45***	0.48***	*ns*	0.68***	*ns*
SURP	*ns*	0.25*	0.28*	0.31**	0.34*	-0.27*	-0.36**	*ns*	*ns*	-0.27*

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N$_{TOTAL}$ = C/N of the soil organic matter; ΔC/N$_{TOTAL}$ = variation on C/N$_{TOTAL}$ after land use change; C$_{FLOM}$ (g kg^{-1}) = C content on the free light organic matter fraction; C$_{FLOM}$ (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC$_{FLOM}$ = variation on C$_{FLOM}$ after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

However, the associations of TOC were not the same throughout the soil profile, specifically regarding to water availability indexes (APPENDIX). At 0-10 and 10-20 cm depth, TOC and C_{FLOM} correlated negatively with MAP and positively with DEF, which translates to an increase on TOC and C_{FLOM} turnover with increasingly water availability. When it gets down to 20-40 and 40-60 cm depth, TOC started to show no significant correlation with MAP or with DEF, but it showed a positive correlation with SURP. At 60-100 cm depth TOC showed a positive correlation with SURP and also with MAP, suggesting that the availability of water at shallow depths (the ones affected by cultivation) is leading to an increase on SOM mineralization, while it is necessary to increase SOM storage deeply. In an experiment by Fontaine et al. (2007) they found that the surface layer of soil was dominated by young fast-cycling C whereas the subsoil was dominated by ancient slow-cycling C, which indicates that the decomposition of SOC is strongly reduced at depth. They proposed that the stability of SOC deeply in the soil reflects a lack of fresh C for microbes. It means that even under favorable conditions of temperature and moisture for microbial activities, SOC from deep soil does not provide enough energy to sustain active microbial populations and thereby the production of enzymes, thus it could reduce or cancel out the effects of climate changes on SOM decomposition.

Soil texture is known for playing an important role on SOC storage, with increasing clay content decreasing C outputs through its stabilizing and protecting effect on SOC (Paul, 1984; Stevenson, 1994; Zinn et al., 2007). Nevertheless, as clay particles have limited adsorbing sites and specific surface area, there is a maximum of C that can associate with it (i.e. C saturation level for the clay particles), which differs between clay types and ecosystems (Hassink, 1997; Hassink and Whitmore, 1997; Six et al., 2002). In general, there are three major SOM stabilization mechanisms (i.e. chemical stabilization, physical protection, and biochemical stabilization) plus the unprotected pool, and each one of them has its own saturation level (Six et al., 2002; Stewart et al., 2008). Regarding to the chemical stabilization mechanism (binding of SOC to clay), Hassink and Whitmore (1997) found a linear relationship between the clay content and the protective capacity of SOM by the soils. Our results also did show a linear relationship between TOC content and clay for all studied depths and also for the whole soil profile (0-100 cm; Figure 4). On the light of the soil C-saturation model our results showed that not only the chemically stabilized pool is decreasing towards deeper soil layers (decreasing slope) but also other pools as well (intercept is also decreasing; Figure 4). But even though other stabilization mechanisms also get weak deeper in the soil, proportionally their contribution increases once the goodness of fit (coefficient of determination – R^2) of the linear regression between TOC content and clay decreases. It also can be seen from the association between TOC stock and clay content still on Figure 4. For 60-100 cm depth and also for the whole soil profile (0-100 cm) the association is no longer linear but rather quadratic. If we can think about clay particles as sources of binding sites for SOC, the increase of clay content would increase linearly the amount of available binding sites, and lastly the amount of SOC associated with it as well. But in order to have a quadratic association of clay content and TOC stock, available binding sites on the clay particles must be left empty, otherwise the relationship would be linear. The derivative of the curve on the 0-100 cm on Figure 4 gives the maximum of the function (77.8 % of clay content), which returns the maximum TOC stock of 157.8 t ha^{-1}. It means that the whole protective effect of clay particles onto SOM is not being completely used on clayey Brazilian soils, especially at deeper layers.

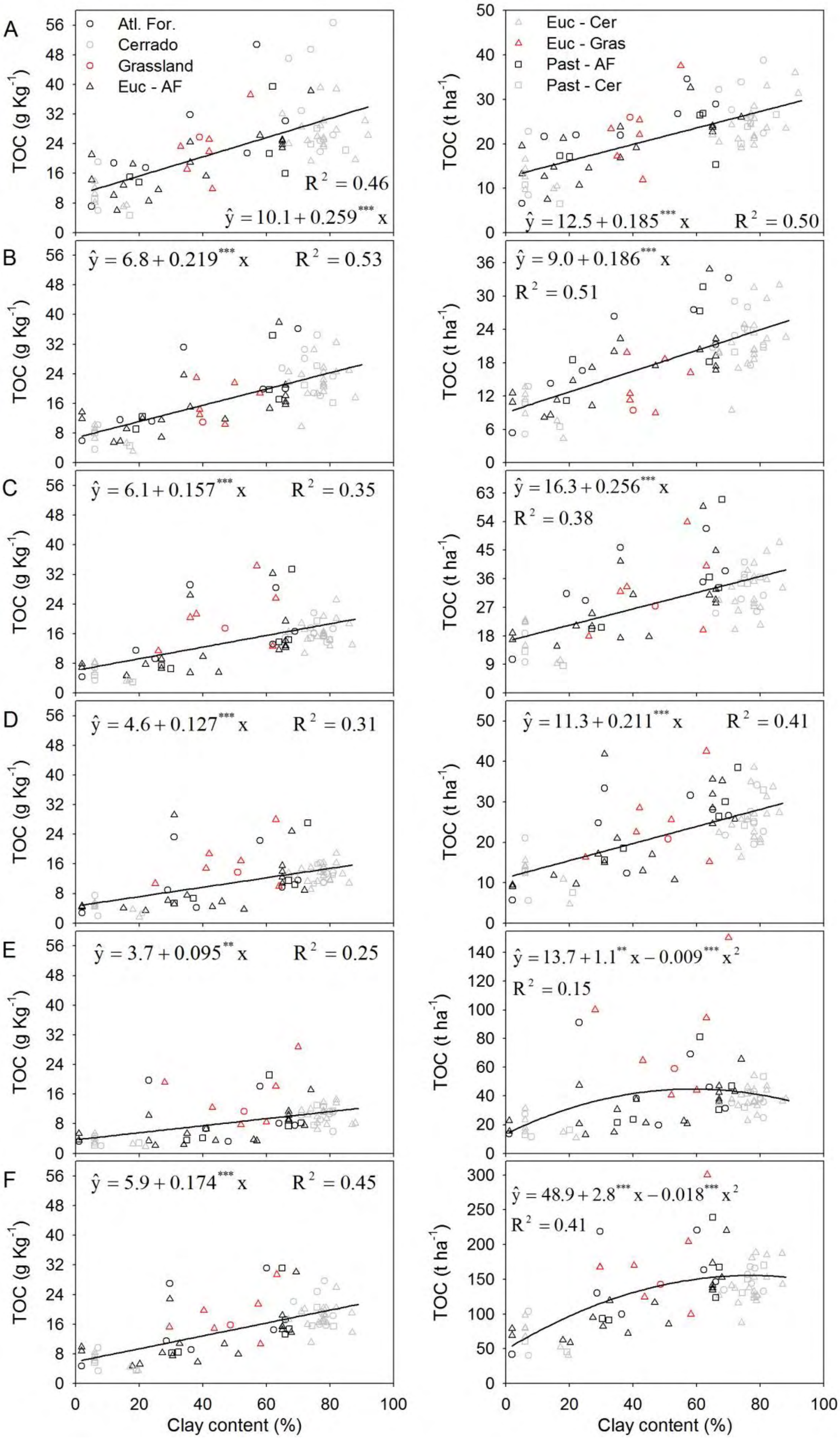

Figure 4. Associations of total organic C (TOC) content (g kg^{-1}) and stock (t ha^{-1}) with clay at each depth. Letters A, B, C, D, E, and F on the left side represent 0-10, 10-20, 20-40, 40-60, 60-100, and 0-100 cm, respectively.

Understanding the implications of this fact can help land owners and Brazilian government on creating policies (e.g. land usage and management) to increase C sequestration in the soils, fulfilling the commitment with cuts on CO_2 emissions and possibly trading C credits on the market. For example, planted forests are known for their potential of acting as a C sink agent (Jackson et al., 2005). As trees represent the vegetation type with the maximum rooting depth in a global scale (Canadell et al., 1996), the potential of planted forests could be even higher than previously thought. The introduction of relatively deeply rooted vegetation into shallow-rooted systems could store C deep in the soil, acting as potential C sink for centuries (Jobbágy and Jackson, 2000), especially if there are compartments of SOM still not saturated. Due to the huge amount of pasture areas in Brazil and to their poorly managed condition, Brazilian government could release technological packages to those farmers, and encourage them to adopt of course, which would include the fertilization of those pastures, the use of the correct species, and the integration of pasture areas with tree species.

A stepwise regression of TOC contents and stocks in the first soil meter and for each studied depth, including clay content, C_{FLOM}, temperature, evapotranspiration, water deficit in the soil, and water surplus are shown in Table 2. The strength of the relationship (R^2) was higher at shallower depths but not lower than 50% in any of them. As the correlation of TOC with climatic characteristics differed among the studied depths, it also happened here. The two shallow depths showed to be dependent on the clay content, C_{FLOM}, and on temperature (0-10 cm) and on clay content, C_{FLOM}, and water deficit (10-20 cm). On the deeper layers (20-40, 40-60, and 60-100 cm) the regressions were more homogenous including only two variables. For the whole soil profile (0-100 cm) a regression model including clay, C_{FLOM} , and DEF explained 64%

of the TOC content (P < 0.001), and a model including just clay and DEF explained 55% of the TOC stock (P < 0.001).

Table 2. Stepwise multiple regression of total organic C (TOC) content and stock with climatic characteristics and clay content for 0-10, 10-20, 20-40, 40-60, 60-100, and 0-100 cm layers.

0-10 cm

$$TOC\ (\text{g.kg}^{-1}) = 21.1 + 0.212^{***}clay + 1.84^{***}C_{FLOM} \qquad R^2 = 0.73$$

$$TOC\ (\text{t.ha}^{-1}) = -11.8 + 0.215^{***}clay + 1.23^{***}C_{FLOM} + 4.06^{***}T_{min} \qquad R^2 = 0.70$$

10-20 cm

$$TOC\ (\text{g.kg}^{-1}) = 6.75 + 0.206^{***}clay + 2.40^{***}C_{FLOM} - 0.028^{***}Def \qquad R^2 = 0.73$$

$$TOC\ (\text{t.ha}^{-1}) = 9.04 + 0.186^{***}clay \qquad R^2 = 0.51$$

20-40 cm

$$TOC\ (\text{g.kg}^{-1}) = 29.8 + 0.149^{***}clay - 0.022^{***}ETP \qquad R^2 = 0.56$$

$$TOC\ (\text{t.ha}^{-1}) = 18.9 + 0.325^{***}clay - 0.051^{***}Def \qquad R^2 = 0.50$$

40-60 cm

$$TOC\ (\text{g.kg}^{-1}) = 26.2 + 0.126^{***}clay - 0.021^{***}ETP \qquad R^2 = 0.56$$

$$TOC\ (\text{t.ha}^{-1}) = 13.5 + 0.283^{***}clay - 0.049^{***}Def \qquad R^2 = 0.59$$

60-100 cm

$$TOC\ (\text{g.kg}^{-1}) = 24.1 + 0.099^{***}clay - 0.020^{***}ETP \qquad R^2 = 0.58$$

$$TOC\ (\text{t.ha}^{-1}) = 15.5 + 0.415^{***}clay + 0.036^{***}SURP \qquad R^2 = 0.50$$

0-100 cm

$$TOC\ (\text{g.kg}^{-1}) = 6.42 + 0.187^{***}clay + 2.36^{***}C_{FLOM} - 0.036^{***}Def \qquad R^2 = 0.64$$

$$TOC\ (\text{t.ha}^{-1}) = 83.8 + 1.54^{***}clay - 0.277^{***}Def \qquad R^2 = 0.55$$

3.3 *Correlations and regressions among SOM variables*

Even though decomposers are proven to be integrators of various element cycles (Manzoni et al., 2010; Plante, 2005) and that their C/N ratio has been observed to be relatively constant across ecosystems ant litter types (Manzoni et al., 2008), our data showed that C/N_{TOTAL} at this regional scale varies (<10 to ~45) and this variation positively correlates with TOC, for all studied soil depths, being stronger correlated at 60-100 cm (Figure 5). Even though the association was not so strong for the shallower depths (10 to 23%) it was significantly positive in all of them, including the whole soil profile (0-100 cm). The same trend held for the changes on TOC (ΔTOC) as well, where it correlated positively with C/N_{TOTAL} (Figure 6). But as we already mentioned, the C/N_{FLOM} which represents the quality of the input material, does not have great influence on TOC. Just for the 0-10 and 10-20 cm layers some weak relationships could be seen (4 and 6%, respectively) but no correlations for the deeper layers or for the whole soil profile (0-100 cm; Figure 5). This fact corroborates with the findings by Manzoni et al. (2008) that the microorganisms in the soil strive to maintain a healthy balance between nitrogen and carbon in their diet, pumping out more carbon as CO_2 when carbon rich materials are added (Manzoni et al., 2008). In other words, it does not matter the quality of the input material but the C/N of the microbial community. This microbial community will drive the C/N of the transformed material (humus) to a value close to its own C/N, no matter the quality of the fresh material. And if that is true, we could wonder that, as the higher TOC correlates positively with C/N_{TOTAL}, the microbial community responsible for the higher TOC and for the higher C/N_{TOTAL} is a different community. And even though we cannot test it here, maybe higher stocks of SOC are associated with specific microbial communities, which should be preferred and stimulated in order to achieve higher C sequestration in the soils.

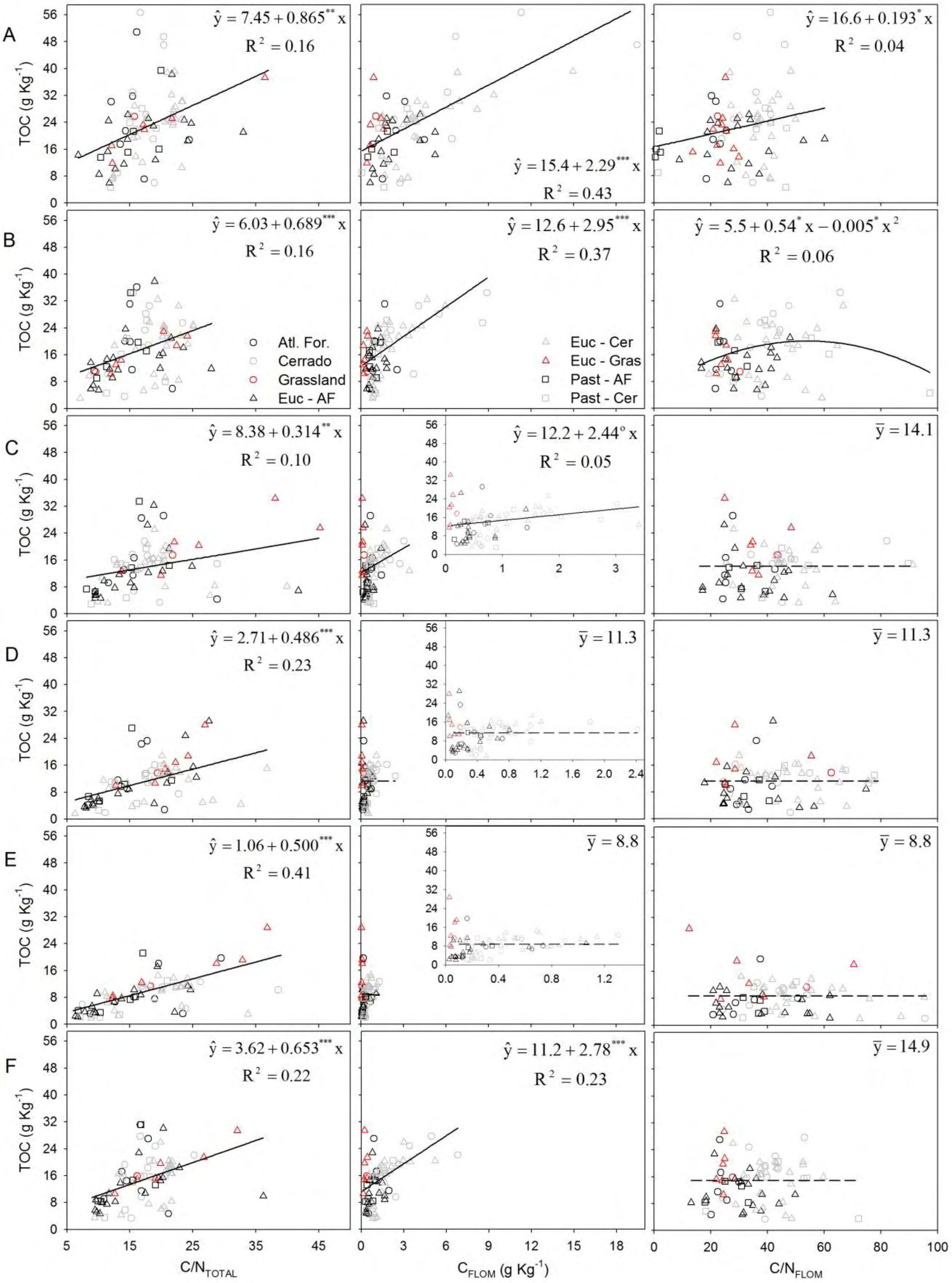

Figure 5. Associations of total organic C (TOC) content with C/N$_{TOTAL}$, C on free light organic matter (C$_{FLOM}$), and C/N$_{FLOM}$. Letters A, B, C, D, E, and F on the left side represent 0-10, 10-20, 20-40, 40-60, 60-100, and 0-100 cm, respectively.

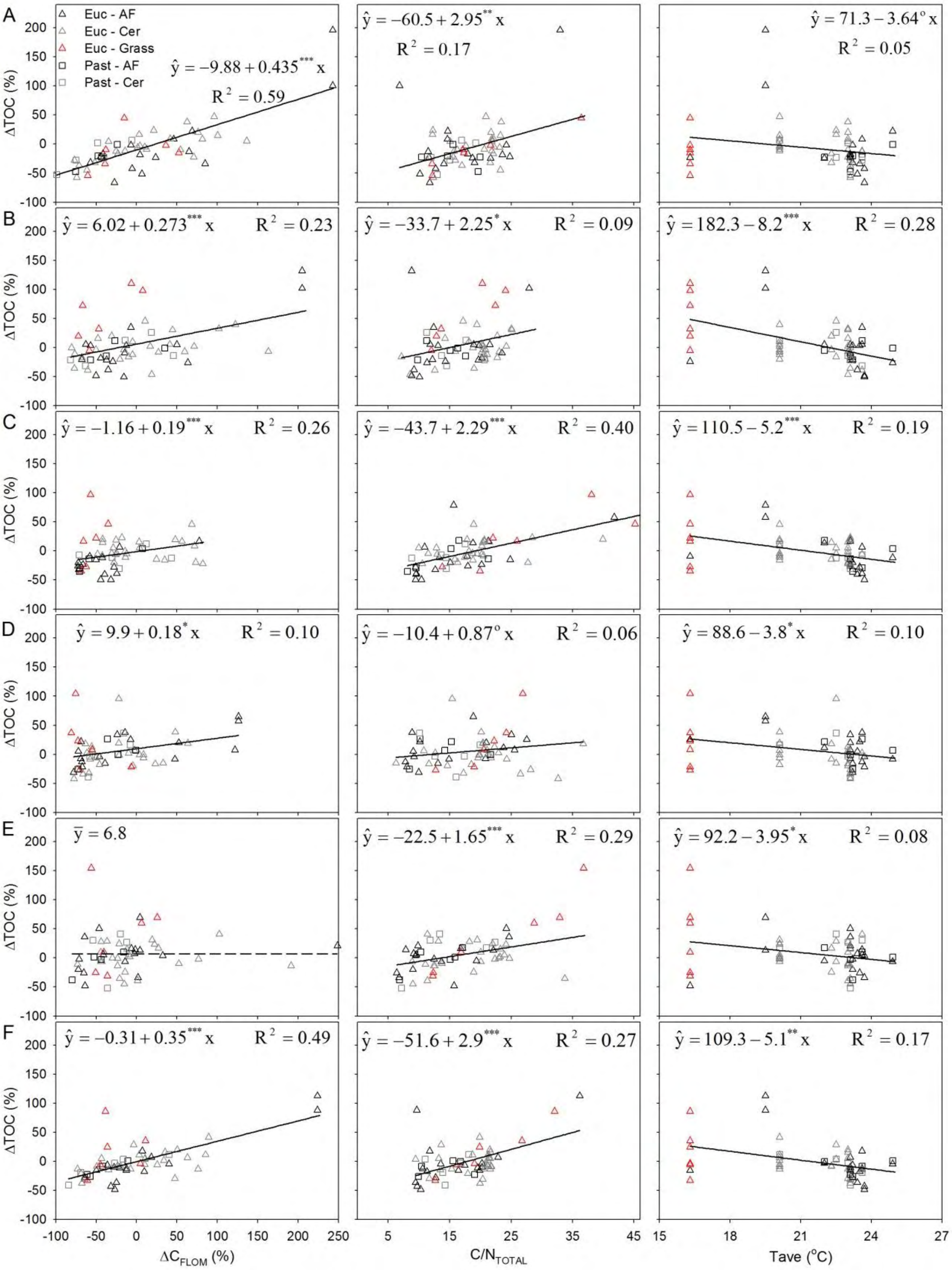

Figure 6. Association of changes on total organic C (ΔTOC) with changes on C of free light organic matter (ΔC$_{FLOM}$), C/N$_{TOTAL}$, and average temperature (T$_{ave}$). Letters A, B, C, D, E, and F on the left side represent 0-10, 10-20, 20-40, 40-60, 60-100, and 0-100 cm, respectively.

Even though Fontaine et al. (2007) proposed that the stability of SOC deeply in the soil reflects a lack of fresh C for microbes, our results did not show it. On the upper layers (0-10, 10-20, and 20-40 cm) and for the whole profile (0-100 cm), TOC correlated positively with C_{FLOM}, but not in deeper layers (Figure 5). If the increase on fresh C should increase SOM mineralization, a decrease on TOC would be expected, but it did not happen. Maybe it could not be seen due to the low amount of C_{FLOM} in those deeper layers. But there is no other way of increasing TOC in deeper layers if not by the increase of fresh C deposition. When looking for the changes on TOC (ΔTOC) and how it behaves in comparison with changes on C_{FLOM} (ΔC_{FLOM}), the same pattern is kept (Figure 6). Higher and positive changes on TOC are driven by high and positive changes on C_{FLOM}, but this association gets weak towards deeper layers (R^2 is decreasing), getting not significant on the 60-100 cm.

The most interesting association of ΔTOC was with average temperature (T_{ave}). Even though TOC has not shown any relation with temperature, the changes on TOC after land use change (ΔTOC) showed a significant negative relation with temperature in all soil depths (Figure 6). And negative changes here translate to a decrease on TOC in comparison with the reference area (native vegetation). As a last result, facing a global climate change the mineralization of SOC after land use change trend to increase. It is known that soil respiration is positively correlated with temperature, which causes an acceleration of the terrestrial carbon cycle in response to global climate change (Bond-Lamberty and Thomson, 2010). But worse than that is the fact that recalcitrant SOC shows a higher sensitivity to increasingly temperature. The lower the rate of microbial respiration, thus the more biogeochemically recalcitrant the organic matter, the greater the temperature sensitivity of soil respiration (Craine et al., 2010).

4. CONCLUSIONS

Our work brings new data on SOC stocks in Brazilian soils, a regional scale work necessary to improve overall estimates of SOC stocks in soils globally. Our results showed that among the two alternative land uses studied (eucalyptus and pasture) pastures are leading to a negative impact on SOC stocks in Brazil. This fact added to the huge amount of degraded pasture areas in Brazil, makes the problem even worst, especially in a CO_2 cutting emissions scenario. We also showed here that clay particles in clayey soils, especially in deeper layers, are still not saturated with SOC, showing that there is still room to sequester C deep in Brazilian soils, helping offset CO_2 emissions. On the light of global climate change, our results showed that increasingly temperature is associated with an increase on SOC mineralization after land use change, making it more difficult to mitigate anthropogenic CO_2 emissions.

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7. APPENDIX

Table A1. Correlation of soil organic matter (SOM) variables with clay content and climatic characteristics for 0-10 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N$_{TOT}$	Δ C/N$_{TOT}$	C$_{FLOM}$ (g kg^{-1})	C$_{FLOM}$ (t ha^{-1})	Δ C$_{FLOM}$	C/N$_{FLOM}$	Δ C/N$_{FLOM}$
TOC t ha^{-1}	0.88^{***}									
Δ TOC	0.29^{*}	0.38^{**}								
C/N$_{TOTAL}$	0.40^{***}	0.44^{***}	0.41^{**}							
Δ C/N$_{TOTAL}$	0.54^{***}	0.59^{***}	0.29^{*}	0.85^{***}						
C$_{FLOM}$ g kg^{-1}	0.65^{***}	0.44^{***}	0.33^{*}	0.29^{*}	*ns*					
C$_{FLOM}$ t ha^{-1}	0.54^{***}	0.42^{***}	0.43^{**}	0.30^{*}	*ns*	0.96^{**}				
Δ C$_{FLOM}$	*ns*	*ns*	0.77^{***}	*ns*	*ns*	0.40^{**}	0.50^{***}			
C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	0.37^{**}	0.39^{**}	ns		
Δ C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.65^{***}	
Clay	0.68^{***}	0.71^{***}	*ns*	0.31^{**}	0.44^{**}	0.35^{**}	0.29^{*}	*ns*	0.37^{**}	*ns*
MAP	-0.31^{**}	*ns*	*ns*	*ns*	*ns*	-0.43^{***}	-0.41^{***}	-0.30^{*}	-0.50^{***}	*ns*
Tave	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Tmax	*ns*	*ns*	-0.27^{*}	*ns*	*ns*	*ns*	*ns*	-0.30^{*}	*ns*	*ns*
Tmin	-0.24^{*}	*ns*	*ns*	-0.28^{*}	-0.32^{*}	*ns*	*ns*	*ns*	*ns*	0.33^{*}
Tamp	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-0.36^{**}	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.29^{*}	*ns*	*ns*
VP	-0.37^{**}	-0.34^{**}	*ns*	*ns*	-0.38^{**}	*ns*	*ns*	0.27^{*}	*ns*	*ns*
ETP	*ns*	*ns*	*ns*	-0.30^{**}	-0.27^{*}	*ns*	*ns*	*ns*	*ns*	0.33^{*}
DEF	0.23^{*}	*ns*	*ns*	*ns*	*ns*	0.40^{**}	0.40^{**}	*ns*	0.60^{***}	*ns*
SURP	*ns*	*ns*	*ns*	*ns*	*ns*	-0.24^{*}	-0.29^{*}	*ns*	-0.28^{*}	*ns*

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N$_{TOTAL}$ = C/N of the soil organic matter; ΔC/N$_{TOTAL}$ = variation on C/N$_{TOTAL}$ after land use change; C$_{FLOM}$ (g kg^{-1}) = C content on the free light organic matter fraction; C$_{FLOM}$ (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC$_{FLOM}$ = variation on C$_{FLOM}$ after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

Table A2. Correlation of soil organic matter (SOM) variables with clay content and climatic characteristics for 10-20 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N$_{TOT}$	Δ C/N$_{TOT}$	C$_{FLOM}$ (g kg^{-1})	C$_{FLOM}$ (t ha^{-1})	Δ C$_{FLOM}$	C/N$_{FLOM}$	Δ C/N$_{FLOM}$
TOC t ha^{-1}	0.91^{***}									
Δ TOC	*ns*	*ns*								
C/N$_{TOTAL}$	0.40^{***}	0.36^{**}	0.30^{*}							
Δ C/N$_{TOTAL}$	*ns*	*ns*	0.34^{*}	0.63^{***}						
C$_{FLOM}$ g kg^{-1}	0.60^{***}	0.43^{***}	*ns*	0.24^{*}	-0.28^{*}					
C$_{FLOM}$ t ha^{-1}	0.53^{***}	0.42^{***}	*ns*	*ns*	-0.32^{*}	0.97^{***}				
Δ C$_{FLOM}$	0.28^{*}	0.34^{*}	0.47^{***}	*ns*	*ns*	0.40^{**}	0.47^{***}			
C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	0.31^{**}	0.31^{**}	*ns*		
Δ C/N$_{FLOM}$	-0.36^{**}	*ns*	-0.32^{*}	*ns*	*ns*	*ns*	*ns*	*ns*	0.71^{***}	
Clay	0.73^{***}	0.72^{***}	*ns*	0.31^{**}	*ns*	0.35^{**}	0.29^{*}	*ns*	0.32^{**}	*ns*
MAP	-0.38^{**}	-0.44^{***}	*ns*	*ns*	0.42^{**}	-0.48^{***}	-0.48^{***}	-0.34^{*}	-0.37^{**}	*ns*
Tave	*ns*	*ns*	-0.53^{***}	*ns*	-0.34^{*}	*ns*	*ns*	*ns*	0.25^{*}	0.51^{***}
Tmax	*ns*	*ns*	-0.54^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.24^{*}	0.55^{***}
Tmin	-0.27^{*}	*ns*	-0.34^{**}	-0.40^{***}	-0.45^{***}	*ns*	*ns*	*ns*	*ns*	0.27^{*}
Tamp	*ns*	*ns*	-0.31^{*}	0.32^{**}	*ns*	*ns*	*ns*	*ns*	*ns*	0.38^{**}
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-0.35^{**}
VP	-0.33^{**}	-0.27^{*}	*ns*	-0.33^{**}	-0.48^{***}	*ns*	*ns*	*ns*	*ns*	*ns*
ETP	-0.24^{*}	*ns*	-0.44^{**}	-0.35^{**}	-0.40^{**}	*ns*	*ns*	*ns*	*ns*	0.38^{**}
DEF	0.23^{*}	0.28^{*}	-0.27^{*}	*ns*	-0.53^{***}	0.43^{***}	0.44^{***}	*ns*	0.61^{***}	*ns*
SURP	*ns*	*ns*	0.43^{**}	*ns*	0.49^{***}	-0.26^{*}	-0.33^{**}	*ns*	*ns*	*ns*

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N$_{TOTAL}$ = C/N of the soil organic matter; ΔC/N$_{TOTAL}$ = variation on C/N$_{TOTAL}$ after land use change; C$_{FLOM}$ (g kg^{-1}) = C content on the free light organic matter fraction; C$_{FLOM}$ (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC$_{FLOM}$ = variation on C$_{FLOM}$ after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

Table A3. Correlation of soil organic matter (SOM) variables with clay content and climatic characteristics for 20-40 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N$_{TOT}$	Δ C/N$_{TOT}$	C$_{FLOM}$ (g kg^{-1})	C$_{FLOM}$ (t ha^{-1})	Δ C$_{FLOM}$	C/N$_{FLOM}$	Δ C/N$_{FLOM}$
TOC t ha^{-1}	0.91^{***}									
Δ TOC	0.53^{***}	0.46^{***}								
C/N$_{TOTAL}$	0.31^{**}	*ns*	0.63^{***}							
Δ C/N$_{TOTAL}$	0.27^{*}	0.30^{*}	0.39^{**}	0.85^{***}						
C$_{FLOM}$ g kg^{-1}	*ns*	*ns*	*ns*	*ns*	*ns*					
C$_{FLOM}$ t ha^{-1}	*ns*	*ns*	*ns*	*ns*	*ns*	0.96^{***}				
Δ C$_{FLOM}$	*ns*	*ns*	0.51^{***}	*ns*	*ns*	0.43^{**}	0.50^{***}			
C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	0.27^{*}	0.27^{*}	*ns*		
Δ C/N$_{FLOM}$	-0.46^{***}	-0.43^{**}	-0.45^{**}	-0.30^{*}	*ns*	*ns*	*ns*	*ns*	0.75^{***}	
Clay	0.59^{***}	0.62^{***}	*ns*	*ns*	*ns*	0.38^{**}	0.26^{*}	*ns*	*ns*	*ns*
MAP	*ns*	*ns*	*ns*	0.28^{*}	*ns*	-0.51^{***}	-0.47^{***}	-0.28^{*}	*ns*	*ns*
Tave	-0.47^{***}	*ns*	-0.44^{**}	-0.46^{***}	*ns*	*ns*	0.28^{*}	*ns*	*ns*	0.37^{**}
Tmax	-0.43^{***}	*ns*	-0.38^{**}	-0.33^{**}	*ns*	*ns*	0.24^{*}	*ns*	*ns*	0.34^{*}
Tmin	-0.50^{***}	-0.25^{*}	-0.48^{***}	-0.59^{***}	-0.39^{**}	*ns*	*ns*	*ns*	*ns*	0.34^{*}
Tamp	*ns*	*ns*	*ns*	*ns*	0.39^{**}	*ns*	*ns*	*ns*	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
VP	-0.40^{***}	-0.36^{**}	*ns*	-0.45^{***}	-0.52^{***}	*ns*	*ns*	*ns*	*ns*	*ns*
ETP	-0.49^{***}	*ns*	-0.52^{***}	-0.59^{***}	-0.32^{*}	*ns*	*ns*	*ns*	*ns*	0.39^{**}
DEF	*ns*	*ns*	*ns*	-0.46^{***}	-0.47^{***}	0.48^{***}	0.47^{***}	*ns*	0.49^{***}	*ns*
SURP	0.33^{**}	*ns*	0.40^{**}	0.49^{***}	0.30^{*}	-0.29^{*}	-0.37^{**}	*ns*	*ns*	-0.27^{*}

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N$_{TOTAL}$ = C/N of the soil organic matter; ΔC/N$_{TOTAL}$ = variation on C/N$_{TOTAL}$ after land use change; C$_{FLOM}$ (g kg^{-1}) = C content on the free light organic matter fraction; C$_{FLOM}$ (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC$_{FLOM}$ = variation on C$_{FLOM}$ after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

Table A4. Correlation of soil organic matter (SOM) variables with clay content and climatic characteristics for 40-60 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N_{TOT}	Δ C/N_{TOT}	C_{FLOM} (g kg^{-1})	C_{FLOM} (t ha^{-1})	Δ C_{FLOM}	C/N_{FLOM}	Δ C/N_{FLOM}
TOC t ha^{-1}	0.87^{***}									
Δ TOC	0.35^{**}	0.27^{*}								
C/N_{TOTAL}	0.26^{*}	*ns*	*ns*							
Δ C/N_{TOTAL}	*ns*	*ns*	0.41^{**}	0.65^{***}						
C_{FLOM} g kg^{-1}	*ns*	*ns*	*ns*	0.40^{**}	*ns*					
C_{FLOM} t ha^{-1}	*ns*	*ns*	*ns*	0.30^{*}	*ns*	0.94^{***}				
Δ C_{FLOM}	*ns*	*ns*	0.32^{*}	*ns*	0.39^{**}	0.50^{***}	0.50^{***}			
C/N_{FLOM}	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		
Δ C/N_{FLOM}	-0.39^{**}	-0.28^{*}	*ns*	-0.30^{*}	*ns*	*ns*	0.32^{*}	*ns*	0.79^{***}	
Clay	0.55^{***}	0.64^{***}	*ns*	*ns*	*ns*	0.38^{**}	0.25^{*}	*ns*	*ns*	*ns*
MAP	*ns*	*ns*	*ns*	*ns*	*ns*	-0.47^{***}	-0.40^{**}	*ns*	*ns*	*ns*
Tave	-0.48^{***}	*ns*	-0.32^{*}	-0.27^{*}	*ns*	*ns*	0.35^{**}	*ns*	*ns*	0.50^{***}
Tmax	-0.42^{***}	*ns*	-0.35^{**}	*ns*	*ns*	*ns*	0.36^{**}	*ns*	*ns*	0.48^{***}
Tmin	-0.52^{***}	-0.29^{*}	*ns*	-0.43^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.36^{**}
Tamp	*ns*	*ns*	-0.26^{*}	*ns*	*ns*	*ns*	0.31^{**}	*ns*	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-0.35^{**}	*ns*	*ns*	-0.40^{**}
VP	-0.41^{***}	-0.41^{***}	*ns*	-0.41^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
ETP	-0.50^{***}	*ns*	*ns*	-0.41^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.48^{***}
DEF	*ns*	*ns*	*ns*	*ns*	-0.32^{*}	0.44^{***}	0.40^{***}	*ns*	0.46^{***}	0.40^{**}
SURP	0.33^{**}	*ns*	*ns*	*ns*	*ns*	-0.26^{*}	-0.32^{**}	*ns*	*ns*	*ns*

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N_{TOTAL} = C/N of the soil organic matter; $\Delta C/N_{TOTAL}$ = variation on C/N_{TOTAL} after land use change; C_{FLOM} (g kg^{-1}) = C content on the free light organic matter fraction; C_{FLOM} (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC_{FLOM} = variation on C_{FLOM} after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

Table A5. Correlation of soil organic matter (SOM) variables with clay content and climatic characteristics for 60-100 cm layer.

	TOC (g kg^{-1})	TOC (t ha^{-1})	Δ TOC	C/N$_{TOT}$	Δ C/N$_{TOT}$	C$_{FLOM}$ (g kg^{-1})	C$_{FLOM}$ (t ha^{-1})	Δ C$_{FLOM}$	C/N$_{FLOM}$	Δ C/N$_{FLOM}$
TOC t ha^{-1}	0.92^{***}									
Δ TOC	0.60^{***}	0.65^{***}								
C/N$_{TOTAL}$	0.39^{**}	0.34^{**}	0.54^{***}							
Δ C/N$_{TOTAL}$	0.27^{*}	0.31^{*}	0.71^{***}	0.83^{***}						
C$_{FLOM}$ g kg^{-1}	*ns*	*ns*	*ns*	*ns*	*ns*					
C$_{FLOM}$ t ha^{-1}	*ns*	*ns*	*ns*	*ns*	*ns*	0.98^{***}				
Δ C$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	0.91^{***}	0.91^{***}			
C/N$_{FLOM}$	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		
Δ C/N$_{FLOM}$	-0.43^{**}	-0.43^{**}	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.60^{***}	
Clay	0.50^{***}	0.32^{**}	*ns*	*ns*	*ns*	0.34^{**}	0.30^{*}	*ns*	*ns*	*ns*
MAP	*ns*	0.32^{**}	*ns*	*ns*	*ns*	-0.37^{**}	-0.34^{**}	*ns*	*ns*	*ns*
Tave	-0.54^{***}	-0.55^{***}	-0.28^{*}	-0.40^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.43^{**}
Tmax	-0.49^{***}	-0.51^{***}	-0.28^{*}	-0.34^{**}	-0.28^{*}	*ns*	*ns*	*ns*	*ns*	0.39^{**}
Tmin	-0.54^{***}	-0.49^{***}	*ns*	-0.43^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.35^{**}
Tamp	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
RH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-0.25^{*}	*ns*
VP	-0.37^{**}	-0.38^{**}	*ns*	-0.23^{*}	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
ETP	-0.56^{***}	-0.52^{***}	*ns*	-0.48^{***}	*ns*	*ns*	*ns*	*ns*	*ns*	0.42^{**}
DEF	*ns*	-0.30^{**}	*ns*	-0.23^{*}	*ns*	0.31^{**}	0.30^{*}	*ns*	0.53^{***}	*ns*
SURP	0.40^{***}	0.52^{***}	0.34^{**}	0.25^{*}	*ns*	*ns*	*ns*	*ns*	*ns*	-0.29^{*}

TOC (g kg^{-1}) = total organic C content; TOC (t ha^{-1}) = total organic C stock; ΔTOC = variation on TOC after land use change; C/N$_{TOTAL}$ = C/N of the soil organic matter; ΔC/N$_{TOTAL}$ = variation on C/N$_{TOTAL}$ after land use change; C$_{FLOM}$ (g kg^{-1}) = C content on the free light organic matter fraction; C$_{FLOM}$ (t ha^{-1}) = C stock on the free light organic matter fraction; ΔC$_{FLOM}$ = variation on C$_{FLOM}$ after land use change; MAP = mean annual precipitation; Tave = average temperature; Tmax = mean maximum temperature; Tmin = mean minimum temperature; Tamp = mean temperature amplitude within a day; RH = relative humidity; VP = vapor pressure; ETP = evapotranspiration; DEF = annual water deficit; SURP = annual water surplus (percolated below the root system).

Table A7. Location, edaphoclimatic characteristics and experiments identification in Atlantic Forest biome.

Treatment	Genetic material	Age (years)	First planting	Rotation	Previous use
Luís Antônio (SP) – 21° 55’ S e 47° 70’ W(1) – Oxisol (2) – Smooth undulated (3) – Cwa(4)					
1 – Atlantic Forest					-
2 – Eucalyptus	Urograndis	2	1973	4th	Atlantic Forest
3 – Pasture					-
Eunápolis (BA) – 16° 28’ S e 39° 15’W(1) – Yellow Ultisol(2) – Flat(3) – Af(4)					
1 – Atlantic Forest					-
2 – Eucalyptus implantation	Urograndis	8	1996	1ª	Pasture
3 – Eucalyptus reform	Urograndis	1	1994	2ª	Pasture
4 – Pasture					-
H.F.B.N.(5) Porto Alegre (RS) – 30° 02’ S e 51° 22’ W(1) –Quartzarenic Entisol(2) – Flat(3) – Cfa(4)					
1 – Atlantic Forest					-
2 – Eucalyptus reform	*E. grandis*	4	1987	2ª	-
H.F.Q.(6) Encruzilhada do Sul (RS) – 30° 52’ S e 52° 52’ W(1) – Ultisol(2) – Smooth undulated(3) – Cfa(4)					
1 – Atlantic Forest					-
2 – Eucalyptus	*E. grandis*	1	1985	2ª	-
Aracruz (ES) – 19° 80’ S e 40° 28’ W(1) – Cohesive yellow Ultisol(2) – Flat(3) – Aw(4)					
1 – Atlantic Forest					-
2 – Eucalyptus	Urograndis	5	1970	5ª	Pasture
3 – Eucalyptus	Urograndis	6	1970	5ª	Pasture
4 – Eucalyptus fertilized and irrigated	Urograndis	6	1970	5ª	Pasture
5 – Eucalyptus	Urograndis	24		2ª	Pasture
6 – Eucalyptus	Urograndis	7	1970	5ª	Pasture
7 – Pasture					Atlantic Forest
Belo Oriente (MG) – 19° 23’ S e 42° 40’ W(1) – Oxisol(2) – Undulated (3) – Aw(4)					
1 – Atlantic Forest					-
2 – Eucalyptus		2	1969	7ª	Pasture
3 – Pasture					-
Virginópolis (MG) – 18° 70’ S e 42° 68’ W(1) – Oxisol(2) – Undulated(3) – Cwa(4)					
1 – Atlantic Forest					-
2 – Eucalyptus		5	1978	4ª	Pasture
3 – Pasture					-

(1) Geographical coordinates. (2) Soil type. (3) Topography. (4) Climate classification according to Köppen. (5) Horto Florestal Barba Negra. (6) Horto Florestal Quitéria.

Table A8. Location, edaphoclimatic characteristics and experiments identification in Cerrado biome (savanna).

Treatment	Genetic material	Age (years)	First planting	Rotation	Previous use
Luís Antônio (SP) – 21° 55’ S e 47° 70’ W(1) – Oxisol(2) – Smooth undulated(3) – Cwa(4)					
1 – Cerrado					
2 – Eucalyptus	Urograndis	2	1973	4ª	Cerrado
3 – Pasture					
Itamarandiba (MG) – 17° 85’ S e 42° 85’ W(1) – Oxisol(2) – Smooth undulated(3) – Cwa(4)					
1 – Cerrado					
2 – Eucalyptus sowing	*E. urophylla*	20	1984	1ª	Cerrado
3 – Eucalyptus	*E. urophylla*	7	1993	1ª	Pasture
4 – Eucalyptus	*E. urophylla*	7	1984	2ª	Pasture
5 – Eucalyptus	*E. urophylla*	7	1973	3ª	Cerrado
6 – Pasture					
Bom Despacho (MG) – 19° 57’ S e 45° 32’ W(1) – Oxisol(2) – Smooth undulated(3) – Cwa(4)					
1 – Cerrado					
2 – Eucalyptus regrowth	*E. grandis*	11	1973	3ª	Pasture
3 – Eucalyptus	*E. grandis*	29	1975	1ª	Pasture
4 – Eucalyptus reform	*E. urophylla*	6	1973	3ª	Pasture
5 – Pasture					
Itacambira (MG) – 17° 05’ S e 43° 30’ W(1) – Oxisol(2) – Flat(3) – Cwa(4)					
1 – Cerrado					
2 – Eucalyptus reform	*E. urophylla*	7	1976	3ª	-
3 – Eucalyptus regrowth (aband)	*E. urophylla*	10	1976	3ª	-
Curvelo (MG) – 18° 83’ S e 44° 67’ W(1) – Oxisol(2) – Flat(3) – Aw(4)					
1 – Cerrado					
2 – Eucalyptus implantation	*E. urophylla*	7	1997	1ª	Pasture
3 – Eucalyptus regrowth	*E. urophylla*	7	1990	2ª	Pasture
Vazante (MG) – 17° 60’ S e 46° 70’ W(1) – Oxisol(2) – Flat(3) – Aw(4)					
1 – Cerrado					
2 – Eucalyptus regrowth	*E. camald.*	7	1980	3ª	Cerrado
3 – Pasture					
João Pinheiro (MG) – 17° 73’ S e 46° 17’ W(1) – Oxisol(2) – Flat(3) – Aw(4)					
1 – Cerrado					
2 – Eucalyptus reform	*E. urophylla*	6	1980	3ª	-
3 – Eucalyptus regrowth	*E. camald.*	7	1989	2ª	-
4 – Pasture					

(1) Geographical coordinates. (2) Soil type. (3) Topography. (4) Climate classification according to Köppen.

Table A9. Location, edaphoclimatic characteristics and experiments identification in the Pampas biome (grassland).

Treatment	Genetic material	Age (years)	First planting	Rotation	Previous use
H. F. C. (1) Triunfo (RS) – 29° 93’ S e 51° 72’ W(2) – Ultisol(3) – Smooth undulated(4) – Cfa(5)					
1 – Grassland					
2 – Eucalyptus	*E. grandis*	10	1994	1ª	-
3 – Eucalyptus	*E. grandis*	13	1991	1ª	-
4 – Eucalyptus	*E. grandis*	22	1982	1ª	-
5 – Eucalyptus reform	*E. grandis*	1	1990	2ª	-
6 – Eucalyptus reform	*E. grandis*	2	1989	2ª	-
7 – Eucalyptus reform	*E. grandis*	4	1987	2ª	-

(1) Horto Florestal Colorado. (2) Geographical coordinates. (3) Soil type. (4) Topography. (5) Climate classification according to Köppen.

Table A10. Density (Dens), texture and average total organic C (TOC), total N (TN), C on free light organic matter (C_{FLOM}) and N on free light organic matter (N_{FLOM}) in Atlantic Forest biome.

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLMO(6)	NFLMO(7)
	cm	g cm^{-3}	........................%........................			...............................t ha^{-1}..........................			
				Luís Antônio (SP)					
1 – Atlantic Forest	0-10	0,96	13	21	66	29,0	2,8	3,0	0,136
	10-20	1,11	11	23	66	21,2	1,7	1,0	0,044
	20-40	1,20	10	21	69	38,4	2,9	1,9	0,076
	40-60	1,09	9	21	70	26,6	2,4	0,8	0,025
	60-100	0,98	10	21	69	31,3	1,9	1,2	0,034
2 – Eucalyptus	0-10	0,97	13	22	65	23,1	1,3	2,1	0,070
	10-20	1,11	13	21	66	18,9	1,0	0,9	0,022
	20-40	1,09	13	21	66	33,7	1,9	1,4	0,044
	40-60	1,09	13	22	65	30,1	1,5	0,9	0,029
	60-100	1,02	11	22	67	40,8	2,8	1,6	0,044
3 – Pasture	0-10	1,23	13	21	66	15,4	0,9	0,8	0,026
	10-20	1,15	13	23	64	18,2	1,2	1,0	0,022
	20-40	1,14	11	22	67	33,1	1,8	1,6	0,021
	40-60	1,11	11	22	67	26,4	1,4	1,2	0,017
	60-100	1,02	11	22	67	30,4	2,3	2,0	0,019
				Eunápolis (BA)					
1 – Atlantic Forest	0-10	1,15	87	1	12	21,6	0,9	5,1	0,247
	10-20	1,23	85	1	14	14,2	0,9	3,1	0,143
	20-40	1,37	79	2	19	31,3	2,0	3,9	0,157
	40-60	1,39	69	2	29	24,8	1,7	2,0	0,074
	60-100	1,43	56	3	41	38,3	3,0	3,7	0,130
2 – Eucalyptus implantation	0-10	1,23	83	2	15	14,7	0,8	2,2	0,247
	10-20	1,39	82	2	16	11,3	0,9	1,0	0,035
	20-40	1,39	75	3	22	21,1	1,7	1,1	0,036
	40-60	1,39	68	3	29	17,1	1,7	0,4	0,017
	60-100	1,31	63	2	35	30,5	3,4	1,0	0,040
3 – Eucalyptus reform	0-10	1,38	80	2	18	21,2	0,9	4,8	0,091
	10-20	1,43	76	3	21	14,7	0,7	1,3	0,052
	20-40	1,45	71	3	26	25,0	1,7	1,1	0,047
	40-60	1,50	63	2	35	20,9	1,6	1,2	0,049
	60-100	1,42	57	2	41	37,5	3,0	1,1	0,049
4 – Pasture	0-10	1,42	81	2	17	17,3	1,2	2,7	0,197
	10-20	1,52	78	3	19	11,2	1,1	1,3	0,047
	20-40	1,46	70	3	27	20,2	2,5	1,2	0,038
	40-60	1,45	60	3	37	18,5	2,2	0,6	0,017
	60-100	1,37	57	3	40	23,7	3,5	0,8	0,019
				H.F.B.N.(8) Porto Alegre (RS)					
1 – Atlantic Forest	0-10	0.93	96	2	2	6,6	0,4	1,4	0,076
	10-20	0.92	96	2	2	5,4	0,2	0,5	0,025
	20-40	1.22	98	0	2	10,6	0,4	0,5	0,020
	40-60	1.03	98	0	2	5,7	0,3	0,3	0,007
	60-100	1.07	99	0	1	13,5	0,6	0,5	0,026
2 – Eucalyptus	0-10	0.93	-	-	-	16,4	1,3	4,9	0,259
	10-20	0.92	-	-	-	11,7	0,9	1,7	0,099
	20-40	1.22	-	-	-	17,8	0,8	2,1	0,124
	40-60	1.03	-	-	-	9,2	0,3	0,6	0,023
	60-100	1.07	-	-	-	19,1	0,5	0,6	0,024

continuing Table A10...

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	FLOM(7)
	cm	g cm^{-3}	....	........%........	....	....	........t ha^{-1}........	....	
				H.F.Q.(9) Encruzilhada do Sul (RS)					
1 – Atlantic Forest	0-10	0.69	53	13	34	21,9	1,4	1,2	0,061
	10-20	0.85	53	13	34	26,3	1,8	1,4	0,061
	20-40	0.79	44	20	36	45,8	2,3	1,0	0,037
	40-60	0.72	53	16	31	33,3	1,9	0,3	0,007
	60-100	1.16	66	11	23	91,2	3,1	0,8	0,020
2 - Eucalyptus	0-10	0.69	36	16	48	16,9	1,5	1,5	0,077
	10-20	0.85	36	16	48	20,0	1,4	1,0	0,046
	20-40	0.79	27	4	69	41,5	2,3	0,4	0,017
	40-60	0.72	24	10	66	41,8	1,5	0,2	0,006
	60-100	1.16	26	11	63	47,4	3,0	0,3	0,011
				Belo Oriente (MG)					
1 – Atlantic Forest	0-10	1,24	40	6	54	26,7	1,9	3,1	0,120
	10-20	1,39	34	7	59	27,5	1,8	1,4	0,057
	20-40	1,33	31	7	62	35,0	2,5	1,9	0,044
	40-60	1,45	31	4	65	28,1	2,1	1,3	0,031
	60-100	1,43	32	4	64	46,2	3,0	4,2	0,083
2 – Eucalyptus	0-10	1,46	35	7	58	32,6	2,2	5,2	0,154
	10-20	1,49	31	8	61	20,3	1,8	2,3	0,091
	20-40	1,28	27	9	64	30,9	2,3	1,1	0,041
	40-60	1,25	26	2	72	25,7	1,7	1,9	0,025
	60-100	1,30	26	2	72	43,1	3,2	3,6	0,069
3 – Pasture	0-10	1,25	34	5	61	26,5	1,7	2,3	0,092
	10-20	1,22	31	8	61	27,3	1,7	1,9	0,084
	20-40	1,27	29	7	64	36,4	2,4	2,0	0,075
	40-60	1,29	28	3	69	30,0	2,1	1,3	0,051
	60-100	1,28	26	3	71	46,9	3,0	2,0	0,064
				Virginópolis (MG)					
1 – Atlantic Forest	0-10	0,68	38	5	57	34,5	2,1	-	-
	10-20	0,92	24	6	70	33,2	2,1	-	-
	20-40	0,91	30	7	63	51,7	3,1	-	-
	40-60	0,71	37	5	58	31,6	1,9	-	-
	60-100	0,96	37	5	58	69,3	3,5	-	-
2 – Eucalyptus	0-10	0,91	21	5	74	26,0	1,2	-	-
	10-20	0,90	30	6	64	34,8	1,8	-	-
	20-40	0,88	33	5	62	58,7	3,1	-	-
	40-60	0,91	28	4	68	35,2	1,5	-	-
	60-100	0,98	20	6	74	65,6	3,4	-	-
3 – Pasture	0-10	1,04	33	5	62	26,8	1,3	-	-
	10-20	0,90	32	6	62	31,7	2,1	-	-
	20-40	0,93	25	7	68	60,9	3,7	-	-
	40-60	0,90	22	5	73	38,5	2,5	-	-
	60-100	1,12	33	6	61	81,1	4,8	-	-
				Aracruz (ES)					
1 – Atlantic Forest	0-10	1,26	73	5	22	22,0	1,6	1,1	0,046
	10-20	1,49	71	5	24	16,6	1,8	1,2	0,045
	20-40	1,58	69	6	25	29,1	2,5	1,6	0,062
	40-60	1,46	58	4	38	12,3	1,5	0,8	0,028
	60-100	1,53	49	3	48	19,7	2,3	0,5	0,017
2 – Eucalyptus	0-10	1,40	56	5	39	21,5	1,6	1,7	0,035
	10-20	1,55	54	4	42	19,9	1,6	0,8	0,019
	20-40	1,55	53	4	43	24,3	2,3	1,2	0,024
	40-60	1,47	47	3	50	13,8	1,6	0,5	0,010
	60-100	1,47	41	2	57	21,6	2,5	0,4	0,007

continuing Table A10...

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	NFLOM(7)
	cm	g cm^{-3}	....	%...........................		..................................t ha^{-1}	....		
				Aracruz (ES)					
3 – Eucalyptus	0-10	1,42	85	2	13	10,1	0,8	0,9	0,033
	10-20	1,50	85	2	13	8,4	0,9	0,8	0,025
	20-40	1,58	82	2	16	14,7	1,5	1,0	0,026
	40-60	1,65	79	2	19	10,7	1,2	0,4	0,009
	60-100	1,66	74	2	24	16,7	2,0	0,5	0,012
4 – Eucalyptus fertilized and irrigated	0-10	1,29	65	5	30	14,4	1,4	0,6	0,021
	10-20	1,51	63	4	33	17,4	1,6	0,6	0,012
	20-40	1,69	62	4	34	26,9	2,9	0,7	0,016
	40-60	1,70	58	3	39	20,9	2,2	0,6	0,016
	60-100	1,71	52	3	45	30,8	3,9	0,4	0,015
5 – Eucalyptus 24 years	0-10	1,45	69	5	26	14,5	1,0	2,0	0,059
	10-20	1,52	70	3	27	17,1	1,3	1,1	0,029
	20-40	1,68	69	4	27	21,0	2,2	1,1	0,028
	40-60	1,72	65	4	31	15,0	1,5	0,3	0,008
	60-100	1,71	63	3	34	14,6	2,3	0,2	0,007
6 – Eucalyptus 7 years	0-10	1,43	74	3	23	10,7	1,1	1,1	0,031
	10-20	1,39	69	4	27	10,2	1,1	0,8	0,021
	20-40	1,69	60	4	36	17,3	1,8	0,9	0,025
	40-60	1,60	53	4	43	12,9	1,4	0,2	0,009
	60-100	1,50	53	3	44	21,1	2,3	0,4	0,008
7 – Pasture	0-10	1,50	77	3	20	17,1	1,6	0,6	0,020
	10-20	1,52	74	5	21	18,5	1,6	0,9	0,028
	20-40	1,58	67	3	30	20,7	2,2	0,5	0,012
	40-60	1,60	64	5	31	15,6	1,5	0,5	0,013
	60-100	1,68	62	3	35	21,6	2,1	0,4	0,011

(1) Soil density by the volumetric ring method. (2) Sieving (Ruiz, 2005). (3) Pippet method, using the Stokes Law to figure out the sedimentation time (Ruiz, 2005). (4) Total organic C. (5) Total N. (6) C on the free light organic matter (FLOM). (7) N on the free light organic matter (FLOM) (8) Horto Florestal Barba Negra. (9) Horto Florestal Quitéria.

Table A11. Density (Dens), texture and average total organic C (TOC), total N (TN), C on free light organic matter (C_{FLOM}) and N on free light organic matter (N_{FLOM}) in Cerrado biome.

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	NFLOM(7)
	cm	g cm^{-3}	....	%.	....	....	t ha^{-1}	....	
				Luís Antônio (SP)					
1 – Cerrado	0-10	1,20	91	2	7	22,8	1,4	7,7	0,320
	10-20	1,41	91	2	7	13,7	1,4	5,0	0,214
	20-40	1,35	92	2	6	18,7	1,4	2,0	0,066
	40-60	1,47	92	2	6	21,0	1,1	3,0	0,113
	60-100	1,46	93	1	6	27,3	1,3	1,5	0,064
2 – Eucalyptus	0-10	1,27	93	1	6	13,3	0,9	2,2	0,063
	10-20	1,41	93	1	6	11,5	0,7	1,2	0,037
	20-40	1,38	92	2	6	20,6	0,9	1,5	0,039
	40-60	1,40	92	2	6	14,0	0,7	0,9	0,021
	60-100	1,40	93	1	6	21,8	1,6	1,3	0,027
3 – Pasture	0-10	1,38	92	2	6	10,7	0,8	0,1	0,055
	10-20	1,44	92	2	6	10,8	0,5	0,9	0,033
	20-40	1,51	93	1	6	13,1	0,9	1,6	0,042
	40-60	1,48	92	2	6	12,8	0,8	1,2	0,040
	60-100	1,48	92	2	6	13,0	1,8	1,0	0,031
				Itamarandiba (MG)					
1 – Cerrado	0-10	0,88	10	9	81	22,4	1,3	2,9	0,072
	10-20	0,88	10	9	81	21,2	1,2	1,9	0,043
	20-40	0,88	10	9	81	30,7	1,7	1,9	0,043
	40-60	0,88	10	9	81	22,6	1,3	1,2	0,022
	60-100	0,88	10	9	81	35,8	1,8	1,9	0,041
2 – Eucalyptus sowing	0-10	0,82	10	8	82	23,6	1,1	7,0	0,171
	10-20	0,82	10	8	82	22,3	1,1	2,7	0,068
	20-40	0,82	10	8	82	35,7	1,7	3,0	0,051
	40-60	0,82	10	8	82	26,9	1,3	2,0	0,040
	60-100	0,82	10	8	82	44,0	1,8	2,3	0,055
3 – Eucalyptus	0-10	0,80	11	11	78	21,7	1,2	3,1	0,084
	10-20	0,80	11	11	78	23,3	1,3	1,8	0,055
	20-40	0,80	11	11	78	36,6	2,1	2,8	0,073
	40-60	0,80	11	11	78	27,0	1,3	1,0	0,019
	60-100	0,80	11	11	78	44,4	2,0	1,5	0,026
4 – Eucalyptus	0-10	0,72	9	9	82	33,0	1,6	5,8	0,216
	10-20	0,72	9	9	82	29,5	1,5	4,3	0,150
	20-40	0,72	9	9	82	44,6	2,4	3,2	0,114
	40-60	0,72	9	9	82	31,3	1,6	1,8	0,060
	60-100	0,72	9	9	82	46,7	2,4	2,2	0,073
5 – Eucalyptus	0-10	0,92	16	6	78	25,7	1,1	5,9	0,141
	10-20	0,92	16	6	78	20,3	1,0	1,6	0,037
	20-40	0,92	16	6	78	29,3	1,6	1,3	0,027
	40-60	0,92	16	6	78	21,3	1,1	1,3	0,032
	60-100	0,92	16	6	78	35,7	1,6	1,3	0,021
6 – Pasture	0-10	0,91	12	12	76	23,9	1,4	2,8	0,071
	10-20	0,91	12	12	76	23,8	1,3	1,6	0,042
	20-40	0,91	12	12	76	34,4	2,0	2,1	0,047
	40-60	0,91	12	12	76	26,4	1,3	1,1	0,024
	60-100	0,91	12	12	76	41,9	2,0	13,7	0,273

continuing Table A11...

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	NFLOM(7)
	cm	g cm^{-3}	..........................%...........................			..................................t ha^{-1}............................			
Bom Despacho (MG)									
1 – Cerrado	0-10	1,19	2	21	77	26,1	1,6	2,9	0,080
	10-20	1,29	1	24	75	24,2	1,3	1,1	0,027
	20-40	1,27	2	23	75	41,2	2,2	1,6	0,033
	40-60	1,27	2	19	79	34,1	2,0	1,5	0,035
	60-100	1,17	2	21	77	42,3	2,6	1,3	0,024
2 – Eucalyptus regrowth	0-10	1,01	3	12	85	29,9	1,4	4,6	0,126
	10-20	1,11	3	19	78	28,1	1,1	1,4	0,032
	20-40	1,23	3	20	77	39,7	2,1	1,3	0,038
	40-60	1,29	3	20	77	36,7	1,6	0,6	0,017
	60-100	1,21	3	20	77	50,2	2,6	0,7	0,015
3 – Eucalyptus	0-10	1,12	2	7	91	36,0	1,5	4,7	0,129
	10-20	1,20	2	12	86	32,0	1,3	2,1	0,051
	20-40	1,22	2	12	86	47,4	2,2	1,6	0,034
	40-60	1,20	2	14	84	33,7	1,7	1,2	0,030
	60-100	1,18	2	10	88	38,1	2,8	2,0	0,042
4 – Eucalyptus reform	0-10	0,92	2	10	88	23,5	1,4	4,5	0,108
	10-20	0,99	2	10	88	22,5	1,4	2,8	0,057
	20-40	1,09	2	11	87	33,0	2,0	2,7	0,048
	40-60	1,15	2	12	86	27,4	1,9	1,5	0,022
	60-100	1,10	2	11	87	36,4	2,9	3,7	0,049
5 – Pasture	0-10	1,35	2	21	77	26,5	1,4	1,5	0,032
	10-20	1,29	1	22	77	20,8	1,5	1,5	0,025
	20-40	1,27	1	22	77	36,4	2,5	1,1	0,020
	40-60	1,33	1	22	77	32,9	1,9	0,8	0,010
	60-100	1,30	2	20	78	53,6	2,4	1,1	0,032
Itacambira (MG)									
1 – Cerrado	0-10	0,67	18	8	74	33,7	1,9	8,6	0,233
	10-20	0,77	20	9	71	25,1	1,6	5,2	0,123
	20-40	0,82	19	9	72	33,9	1,9	4,7	0,116
	40-60	0,85	17	8	75	24,9	1,3	2,8	0,070
	60-100	0,86	18	6	76	37,5	1,9	3,2	0,067
2 – Eucalyptus reform	0-10	0,63	15	8	77	29,6	1,2	6,0	0,114
	10-20	0,77	16	9	75	23,3	1,2	6,6	0,101
	20-40	0,81	14	6	80	22,8	1,1	4,6	0,063
	40-60	0,82	13	7	80	19,9	0,5	2,2	0,031
	60-100	0,84	13	8	79	28,0	1,1	2,9	0,054
3 – Eucalyptus regrowth (abandoned)	0-10	0,78	28	7	65	20,3	0,9	5,0	0,125
	10-20	0,89	25	8	67	18,5	0,9	2,9	0,073
	20-40	0,92	23	5	72	30,3	1,6	2,6	0,056
	40-60	0,91	22	4	74	25,8	1,0	1,7	0,037
	60-100	0,89	21	5	74	44,1	2,1	2,8	0,085
Curvelo (MG)									
1 – Cerrado	0-10	0,99	11	12	77	24,6	1,2	2,3	0,081
	10-20	0,97	11	12	77	17,5	1,0	1,1	0,033
	20-40	0,93	9	12	79	25,6	1,5	0,9	0,026
	40-60	0,90	9	13	78	19,5	1,1	0,8	0,019
	60-100	0,88	8	12	80	37,5	2,2	2,0	0,044
2 – Eucalyptus implantation	0-10	1,05	3	21	76	25,7	1,7	1,5	0,049
	10-20	1,07	3	19	78	20,2	1,4	0,6	0,020
	20-40	1,09	2	18	80	26,9	2,1	0,7	0,018
	40-60	1,03	2	18	80	20,7	1,9	0,4	0,010
	60-100	0,97	1	20	79	33,2	3,0	0,5	0,012

continuing Table A11...

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	NFLOM(7)
	cm	g cm^{-3}	..........................%..........................			..................................t ha^{-1}............................			
				Curvelo (MG)					
3 – Eucalyptus regrowth	0-10	0,91	12	14	74	19,1	1,2	3,9	0,111
	10-20	0,97	11	18	71	9,4	1,1	1,3	0,040
	20-40	1,01	12	16	72	19,9	1,9	1,7	0,039
	40-60	1,05	11	15	74	16,3	1,4	1,0	0,017
	60-100	0,99	10	14	76	22,7	2,0	2,1	0,044
				Vazante (MG)					
1 – Cerrado	0-10	0,84	11	18	71	27,6	1,8	5,1	0,142
	10-20	0,95	11	17	72	29,0	1,6	3,9	0,081
	20-40	1,16	9	16	75	37,3	3,0	4,8	0,096
	40-60	1,14	7	15	78	25,1	2,1	2,0	0,082
	60-100	1,05	7	12	81	24,7	3,3	1,8	0,053
2 – Eucalyptus	0-10	0,97	14	9	77	24,2	1,5	3,8	0,141
	10-20	0,99	13	11	76	17,8	1,4	1,5	0,030
	20-40	0,97	11	11	78	35,2	2,7	2,4	0,053
	40-60	0,98	11	13	76	25,6	2,0	1,8	0,024
	60-100	0,97	11	13	76	34,7	3,1	3,6	0,038
3 – Pasture	0-10	1,03	12	14	74	20,0	1,3	1,3	0,031
	10-20	1,06	12	16	72	19,9	1,4	1,4	0,026
	20-40	1,00	11	15	74	34,6	2,5	1,4	0,015
	40-60	0,96	11	14	75	24,2	1,9	0,8	0,011
	60-100	0,94	11	14	75	32,2	2,7	0,8	0,008
				João Pinheiro (MG)					
1 – Cerrado	0-10	1,41	92	1	7	8,5	0,4	3,9	0,111
	10-20	1,43	93	1	6	5,2	0,4	1,2	0,033
	20-40	1,45	93	1	6	9,7	0,5	1,8	0,040
	40-60	1,47	92	1	7	5,5	0,5	1,1	0,024
	60-100	1,45	91	1	8	11,5	1,1	1,9	0,020
2 – Eucalyptus reform	0-10	1,40	82	3	15	9,8	0,8	4,0	0,097
	10-20	1,48	83	1	16	7,5	0,4	1,4	0,026
	20-40	1,48	82	2	16	9,3	0,8	1,4	0,027
	40-60	1,45	80	2	18	10,8	0,7	0,9	0,013
	60-100	1,40	80	2	18	14,7	1,1	1,2	0,014
3 – Eucalyptus regrowth	0-10	1,37	81	3	16	10,4	0,9	4,7	0,115
	10-20	1,43	80	2	18	4,3	0,6	1,1	0,019
	20-40	1,43	81	2	17	10,2	1,0	1,9	0,029
	40-60	1,44	78	2	20	4,7	0,8	1,5	0,015
	60-100	1,37	76	2	22	10,7	1,2	1,1	0,015
4 – Pasture	0-10	1,48	80	3	17	6,5	0,5	2,3	0,049
	10-20	1,45	79	4	17	6,5	0,6	1,5	0,015
	20-40	1,46	79	3	18	8,5	1,0	2,6	0,021
	40-60	1,42	77	2	21	7,6	0,8	1,0	0,007
	60-100	1,38	77	3	20	16,2	1,2	1,6	0,013

(1) Soil density by the volumetric ring method. (2) Sieving (Ruiz, 2005). (3) Pippet method, using the Stokes Law to figure out the sedimentation time (Ruiz, 2005). (4) Total organic C. (5) Total N. (6) C on the free light organic matter (FLOM). (7) N on the free light organic matter (FLOM)

Table A12. Density (Dens), texture and average total organic C (TOC), total N (TN), C on free light organic matter (C_{FLOM}) and N on free light organic matter (N_{FLOM}) in Pampas biome (grassland).

Treatment	Depth	Dens. (1)	Sand (2)	Silt (3)	Clay (3)	TOC (4)	TN (5)	CFLOM(6)	NFLOM(7)
	cm	g cm^{-3}	..........................%..........................			.................................t ha^{-1}............................			
				H. F. C. (8) Triunfo (RS)					
1 – Grassland	0-10	1.01	45	15	40	25,9	1,7	1,0	0,047
	10-20	0.86	45	15	40	9,4	1,0	0,4	0,012
	20-40	0.78	38	15	47	27,4	1,3	0,3	0,007
	40-60	0.76	37	12	51	20,8	1,1	0,3	0,004
	60-100	1.31	34	13	53	59,1	3,2	0,3	0,006
2 – Eucalyptus	0-10	0.79	47	14	39	17,2	1,4	0,6	0,029
	10-20	0.90	47	14	39	12,4	0,9	0,2	0,008
	20-40	0.96	47	17	36	31,9	1,2	0,1	0,003
	40-60	0.94	43	16	41	22,5	1,1	0,1	0,004
	60-100	0.77	34	14	52	40,4	3,3	0,2	0,009
3 – Eucalyptus	0-10	0.69	44	17	39	22,0	1,3	1,6	0,076
	10-20	0.72	44	17	39	11,2	0,9	0,1	0,004
	20-40	0.99	54	20	26	17,9	0,9	0,1	0,003
	40-60	1.08	59	16	25	16,2	0,9	0,3	0,010
	60-100	1.17	52	20	28	100,0	3,0	0,4	0,015
4 – Eucalyptus	0-10	1.03	40	10	50	25,3	1,2	1,4	0,059
	10-20	1.03	40	10	50	18,6	0,8	0,4	0,019
	20-40	0.84	26	11	63	40,0	0,9	0,2	0,004
	40-60	1.01	35	13	52	25,6	1,2	0,1	0,003
	60-100	0.83	21	16	63	94,4	3,3	0,4	0,005
5 – Eucalyptus reform	0-10	1.01	30	12	58	37,5	1,0	0,9	0,035
	10-20	1.04	30	12	58	16,2	0,7	0,1	0,005
	20-40	0.77	35	8	57	53,8	1,4	0,1	0,005
	40-60	0.79	28	9	63	42,5	1,6	0,1	0,002
	60-100	0.71	19	11	70	150,3	4,1	0,1	0,012
6 – Eucalyptus reform	0-10	0.87	45	17	38	23,4	1,4	0,6	0,027
	10-20	0.84	45	17	38	19,8	1,0	0,4	0,016
	20-40	0.96	44	18	38	33,4	1,5	0,2	0,004
	40-60	0.93	42	16	42	28,5	1,2	0,1	0,001
	60-100	0.83	41	16	43	64,7	3,8	0,2	0,006
7 – Eucalyptus reform	0-10	1.07	44	9	47	11,9	1,0	0,4	0,018
	10-20	1.02	44	9	47	8,9	0,7	0,2	0,007
	20-40	0.88	27	11	62	19,8	1,4	0,1	0,003
	40-60	0.77	19	17	64	15,1	1,2	0,1	0,003
	60-100	0.84	19	21	60	44,0	3,6	0,2	0,004

(1) Soil density by the volumetric ring method. (2) Sieving (Ruiz, 2005). (3) Pippet method, using the Stokes Law to figure out the sedimentation time (Ruiz, 2005). (4) Total organic C. (5) Total N. (6) C on the free light organic matter (FLOM). (7) N on the free light organic matter (FLOM). (8) Horto Florestal Colorado.

GENERAL CONCLUSIONS

Our work contributes in different ways to a better understanding of the global C cycle. Our new global estimates for nutrient concentrations and resorption efficiencies should improve models that explicitly represent the cycling of C and nutrients. Also, it should allow the modeling community to represent more explicitly the coupling of other nutrient cycles within plants and ecosystems. In our work on the thermodynamic study of humic substances we employed successfully a relatively new technique, showing new data about humic substance’s structure and behavior. It should help understand the fate of different compounds on the environment and also the process of C stabilization as SOC. In our last work on SOC storage in Brazilian soils we quantified the amount of SOC under native vegetation and eucalyptus and pasture plantations in three major Brazilian biomes. As important outcomes of this analysis we showed that clay particles in Brazilian clayey soils, especially in deeper layers, are still not saturated with SOC, showing that there is still room to sequester C, helping offset CO_2 emissions. Also, on the light of global climate change, our results showed that increasingly temperature is associated with an increase on SOC mineralization after land use change, making it more difficult to remediate.

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