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PhD thesis

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Soil organic carbon responses to forest expansion on mountain grasslands

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Summary

English

Grassland abandonment followed by progressive forest expansion is the dominant land-use change in the European Alps. Contrasting trends in soil organic carbon (SOC) stocks have been reported for mountainous regions following forest expansion on grasslands. Moreover, its effects on SOC properties involved into long-term stability are largely unknown. The aim of this PhD thesis was to explore changes in: (i) SOC stocks; (ii) physical SOC fractions and their sensitivity to SOC changes; and (iii) labile carbon (C) in soil and fractions following forest expansion on mountain grasslands.

A land-use gradient located in the Southern Alps (Italy) was examined, comprising managed grassland, two transitional phases in which grassland abandonment led to colonization by *Picea abies* (L.) Karst., and old forest dominated by *Fagus sylvatica* L. and *P. abies*. Organic and mineral soil layers were collected within three plots for each successional stage. Soil samples were fractionated according to: (i) aggregate size fractionation, separating aggregates through wet-sieving, and (ii) size-density fractionation, separating stable aggregates from particulate organic matter (POM) non-occluded within aggregates. Changes in labile soil C were assessed by analysis of carbohydrate monomers and by thermal analysis of soil and size-density fractions.

Our study showed that mineral SOC stocks were lower in early-stage and old forest (-28% in the top 10 cm of mineral soil) than in managed and abandoned grassland. The SOC accumulation within the organic layers following forest establishment could not fully compensate the mineral SOC stock difference between forest and grassland successional stages.

The dimension of aggregates assessed by aggregate size fractionation tended to increase, while SOC allocation to stable aggregates assessed by size-density fractionation decreased following conversion of grassland to forest (e.g from 81 to 59% in the 0-5 cm layer). Higher SOC allocation to POM fraction in the mineral soil, together with changed carbon distribution between mineral and organic soil layers, suggest an overall decrease in physical SOC protection and a concomitant shift to unprotected SOC fractions. The size-density fractions showed higher sensitivity to SOC changes than aggregate size fractions and therefore may be better suited for the detection of SOC changes.

Carbohydrate and thermal analyses indicated higher shares of labile soil C in grassland abandoned since 10 years than in managed grassland and forest successional stages. Ratios of

microbially to plant-derived carbohydrates decreased from grassland to forest successional stages, and corresponded to decreased SOC allocation to stable aggregates.

Forest expansion on mountain grasslands caused a decrease in SOC stocks within the mineral soil and in physically protected SOC fractions, which can be explained by lower accumulation of binding agents of microbial origin. This can have implications for the accumulation of atmospheric CO_2 in soil and for the susceptibility of SOC to external disturbances such as management and environmental changes.

Danish

Græsarealer under naturlig tilgroning med skov er den dominerende arealanvendelsesændring i de europæiske Alper. Formålet med denne PhD-afhandling har været at undersøge ændringer i: (i) puljer af kulstof (C); (ii) fordelingen af C på forskellige fraktioner af organisk stof; og (iii) det labile kulstof efter tilgroning af græsarealer i bjergområder.

En kombineret successions- og arealanvendelsesgradient bestående af permanent fodergræs to overgangsfaser under tilgroning med *Picea abies* (L.) Karst., og gammel skov domineret a *Fagus sylvatica* L. og P. *abies* blev undersøgt i de sydlige Alper (Italien). Det organiske lag og mineraljord blev indsamlet i tre delområder for hvert af successionsstadierne. Fordelingen af C på forskellige fraktioner af organisk stof blev karaktereiseret efter to metoder (aggregatstørrelses- og partikelstørrelse-densitetsfraktionering). Ændringer i labilt C blev vurderet ud fra kulhydratanalyse og termisk analyse af jordprøver og fraktioner.

Vores studie viste, at mineraljordens C-puljer var lavere i tidlige successionsstadier og i gammel skov end i de drevne og opgivne græslandsarealer. Kulstof akkumuleringen i de organiske lag efter etablering af skov kunne ikke fuldstændigt kompensere for faldet i den mineralske C-pulje fra græs til skov.

Størrelsen af aggregater havde en tendens til at øges, mens det C, der var i stabile aggregater faldt gradvist efter tilgroning af græsarealerne med skov. Den højere mængde C i fraktionen af partikulært organisk stof i mineraljorden antyder desuden en generel reduktion i fysisk beskyttelse af jordens C pulje. Størrelses-densitetsfraktionerne viste højere sensitivitet i forhold til ændringer i arealanvendelse end aggregatstørrelsesfraktionering, og er måske således bedre egnet som indikator for ændringer i C puljens sammensætning.

Kulhydratanalyser og termisk analyse indikerede en højere andel af labilt C i græs, der blev opgivet for 10 år siden, end i de drevne græsarealer og efterfølgende skovstadier. Forholdet mellem mikrobielt og plante afledte kulhydrater faldt fra græs til skovsuccession stadierne og gammel skov, og korresponderer til faldet i C i stabile aggregater.

Tilgroning græsarealer med skov resulterede i en lavere kapacitet for at lagre C i mineraljorden og en mindsket fysisk beskyttelse belyst ved analyse af kulstoffraktioner. Dette kan forklares ved mindre akkumulering af kulstofforbindelser af mikrobiel oprindelse, der fremmer dannelsen af stabile aggregater. Resultaterne peger på, at skovtilgroning af græsarealer kan have implikationer for akkumuleringen af atmosfærisk CO_2 i jorden samt fordelingen til jordens forskellige C fraktioner. Tilgroning af græsarealer kan således antages at øge C puljens følsomhed overfor eksterne påvirkninger som f.eks. ændret forvaltning og klima.

Italian

L’abbandono dei prati e la loro colonizzazione da parte del bosco costituisce uno dei principali cambiamento d’uso del suolo nelle zone alpine. Tuttavia, gli effetti dell’espansione delle foreste sui prati abbandonati a livello di carbonio organico del suolo sono ancora poco conosciuti. Questa tesi di dottorato ha avuto quindi come obiettivo lo studio dei cambiamenti di carbonio organico in termini stock e di frazioni fisiche e chimiche, in seguito all’espansione delle foreste su prati montani.

L’area studio (Trentino, Italia) è costituita da un prato montano, due fasi di transizione in cui l’abbandono del prato ha portato all’espansione dell’abete rosso (*Picea abies* (L.) Karst), ed una foresta dominata da faggio (*Fagus sylvatica* L.) ed abete rosso. Campioni di suolo sono stati prelevati dagli orizzonti organici e minerali all’interno di tre aree per ogni fase della successione. I campioni di suolo minerale sono stati analizzati per mezzo di tecniche di frazionamento fisico degli aggregati, tramite analisi dei carboidrati e di proprietà termiche.

Il nostro studio ha mostrato come lo stock di carbonio organico nel suolo minerale sia diminuito durante la successione naturale da prato a foresta. Inoltre, l’accumulo di carbonio negli strati organici non ha potuto compensare completamente il minore contenuto di carbonio nel suolo minerale della foresta rispetto al prato.

Mentre la dimensione degli aggregati è aumentata durante la successione da prato a foresta, il carbonio organico contenuto negli aggregati stabili è diminuito. L’incremento di carbonio organico in frazioni non protette all’interno degli aggregati ha comportato una riduzione della protezione fisica del carbonio nel suolo minerale.

Le analisi dei carboidrati e delle proprietà termiche del suolo hanno mostrato come una maggiore quota del carbonio organico complessivo sia presente in forma di carbonio “labile” nel prato abbandonato rispetto alle altre fasi di successione. Il rapporto tra carboidrati di origine microbica e vegetale è diminuito con il passaggio da prato a foresta, in maniera simile alla diminuzione degli aggregati stabili del terreno.

L’espansione forestale su prati montani ha quindi causato una riduzione degli stock di carbonio organico nel suolo minerale e negli aggregati stabili, probabilmente a causa di una riduzione di carboidrati o di altri composti di origine microbica coinvolti nella stabilizzazione degli aggregati. Questo può avere importanti ricadute a livello di stabilità del carbonio organico nel suolo e della sua vulnerabilità nei confronti di cambiamenti gestionali o ambientali.

List of papers

This PhD thesis includes the following original research papers:

PAPER I

Guidi C, Vesterdal L, Gianelle D, Rodeghiero M (2014). Changes in soil organic carbon and nitrogen following forest expansion on grassland in the Southern Alps. *Forest Ecology and Management* 328: 103-116. http://dx.doi.org/10.1016/j.foreco.2014.05.025

PAPER II

Guidi C, Magid J, Rodeghiero M, Gianelle D, Vesterdal L (2014). Effects of forest expansion on mountain grassland: changes within soil organic carbon fractions. *Plant and Soil* 385 (1-2): 373-387. http://dx.doi.org/10.1007/s11104-014-2315-2

PAPER III

Guidi C, Rodeghiero M, Magid J (2014). Towards a simple and sensitive size-density fractionation approach for determining changes in soil organic matter. Manuscript under review in *Geoderma*

PAPER IV

Guidi C, Cannella D, Leifeld J, Rodeghiero M, Magid J, Gianelle D, Vesterdal L. Carbohydrates and thermal properties indicate changes in stable aggregate carbon following forest colonization of mountain grassland. Manuscript under review in *Soil Biology and Biochemistry*

Things shuld be made as simple as possible,
but not simpler

Albert Einstein

Introduction

Land-use changes as drivers of global climate

Land-use activities have transformed land surface since thousands of years, with the ultimate goal of satisfying human needs of food and other resources. Land-use changes (LUCs) are global drivers of modifications in land cover, ecosystem functions and climate (Foley et al., 2005). Global climate is modified by LUCs through emissions of greenhouse gases (CO_2, CH_4, N_2O), but also through changes in surface-energy budgets and water balance (Pielke et al., 2002; Pongratz et al., 2010; Schwaiger and Bird, 2010). The LUCs represent the second largest contributor to atmospheric CO_2 emissions after fossil fuel combustion (van der Werf et al., 2009), and around 12.5% of anthropogenic carbon (C) emissions originated from LUCs from 1990 to 2010 (Houghton et al., 2012). Even though terrestrial ecosystems represent a net C sink (Janssens et al., 2003; Canadell et al., 2007), anthropogenic CO_2 emissions have grown faster than terrestrial C sinks (Le Quéré et al., 2009), leading to the highest CO_2 concentrations in the atmosphere ever recorded (Le Quéré et al., 2014). Soils are the largest terrestrial pool of organic C (Batjes, 1996), and variations in soil C storage as a consequence of LUCs can have a significant impact on atmospheric CO_2 levels (Lal, 2004). Therefore, it is crucial to quantify the effect of LUCs on soil organic carbon (SOC), but this can be complicated due to slow changes in SOC stocks and to spatial heterogeneity in soil (Post et al., 2001).

Forest expansion on grasslands: effects on SOC stocks

Forest expansion on former grasslands is widespread in many regions, as semi-arid grasslands in Great Plains of North America (Hibbard et al., 2003), tropical pastures of Central America (Neumann-Cosel et al., 2011) and mountainous grasslands in Europe (Gellrich et al., 2007; Zimmermann et al., 2010). This grassland-to-forest transition is mainly attributed to modification in the management practices, even though climate change may facilitate forest expansion (Archer et al., 1995; Briggs et al., 2005; Gehrig-Fasel et al., 2007). Large areas of agricultural lands have been abandoned worldwide in the last decades due to socio-economic changes (MacDonald et al., 2000; Brown et al., 2005; Grau and Aide, 2008; Zhang et al., 2010; Fuchs et al., 2013), followed by tree establishment as a result of natural succession (Haugo et al., 2013). However, the expansion of trees and woody shrubs may be considered as an unintended and indirect effect of land management, and therefore it was not included in any of the global estimates of net carbon emissions from LUC (Houghton et al., 2012). Moreover, the quantification of the areal extent of forest expansion and its effect on net carbon emissions is highly problematic (Asner et al., 2003). The increase in carbon stored in aboveground biomass following forest establishment can be offset by SOC losses, as reported in case of woody plant invasion of grasslands in the southwestern USA (Jackson et al., 2002).

The abandonment of grasslands followed by forest expansion is the dominant LUC in mountainous areas of Europe (Tappeiner et al., 2008; Zimmermann et al., 2010). For example, 41% of farms and about 20% of the usable agricultural land were abandoned from 1980 to 2000 (Tappeiner et al., 2008). Despite the recent and current increase in forest area due to grassland abandonment, only few studies have investigated the effect of this LUC on SOC stocks in mountainous temperate regions, where changes in SOC are expected to occur at lower rates compared with warmer, lowland regions (Olson, 1963; Trumbore, 1993; Laganiere et al., 2010). Contrasting direction and magnitude of changes in SOC stocks have been reported following forest colonization of mountain grasslands as several factors can affect SOC changes, such as climate, management history, tree species colonizing the grassland, soil type and time since abandonment. A decrease in mineral SOC stocks was reported by Thuille et al. (2000) and Thuille and Schulze (2006) for coniferous forest expansion and by Alberti et al. (2008) for deciduous forest colonization. Other studies have reported an increase or no change in mineral SOC stock, such as following shrub encroachment into mountain grasslands of the Iberian peninsula (Montane et al., 2007) and conifer forest development in the Swiss Alps (Risch et al., 2008).

Changes in SOC stocks resulting from forest expansion on grasslands are controlled by modifications in the balance between C inputs and outputs. Not only may the overall quantity of C inputs change but also the aboveground vs. belowground distribution of these inputs. Grasslands are characterized by a large belowground allocation of C, while forests allocate more C inputs aboveground through litterfall (Jobbagy and Jackson, 2000; Guo et al., 2008). Aboveground inputs decomposing in organic layers may be transferred into the mineral soil by leaching of dissolved organic C (Kalbitz and Kaiser, 2008; Sanderman and Amundson, 2009) and by transfer of particulate organic matter (POM) for example through faunal activity (Van Delft et al., 1999). On the contrary belowground inputs (i.e. roots and exudates) are directly incorporated in the mineral soil and thus their chances of being transformed into SOC are greater than aboveground residues (Rasse et al., 2005). Decomposition rates of aboveground and belowground inputs may also change after forest expansion, due to modifications in the litter quality (e.g. carbohydrates and lignin content), decomposer communities and microclimatic conditions (Nosetto et al., 2005; Macdonald et al., 2009; Hiltbrunner et al., 2013).

Forest expansion on grasslands: effects on SOC stability

Changes in land use can affect not only the amount of C stored in soil but also its stability, in terms of protection against mineralization (von Lützow et al., 2006; Kögel-Knabner et al., 2008). The responses of SOC stability to forest expansion on mountain grasslands are largely unknown, and changes in SOC stability may follow different directions from trends in bulk SOC stocks (Creamer et al. 2011).

The overall SOC stability results from the synergy of various stabilization mechanisms such as: (i) selective preservation due to recalcitrance, i.e. intrinsic molecular characteristics of organic components; (ii) spatial inaccessibility of organic matter (OM) against decomposers by occlusion and hydrophobicity; and (iii) interactions of OM with mineral surfaces and metal ions (von Lützow et al., 2006). However, the importance of recalcitrance as the major stabilization mechanism has recently been questioned (von Lützow et al., 2006; Dungait et al., 2012; Cotrufo et al., 2013). In fact, evidence has shown that any OM component, even the most recalcitrant outside the soil, can be decomposed by microorganisms (Gooday, 1990; Kögel-Knabner, 2000; Hamer et al., 2004). Compounds typically considered as recalcitrant, first of all lignin and lipids, were shown to turn over even more rapidly than total SOC (Marschner et al., 2008; Dungait et al., 2012), while easily decomposable compounds such as simple sugars, amino acids and microbial polysaccharides were found to be abundant in old SOC, in apparent contrast to their labile nature (Kiem and Kögel-Knabner, 2003; Derrien et al., 2006; Rumpel et al., 2010). Intrinsic recalcitrance is considered to be important mainly in initial decomposition stages while stability in advanced stages of decomposition is rather determined by spatial inaccessibility and organo-mineral associations as well as by interactions with the biotic and abiotic environment (von Lützow et al., 2006; Schmidt et al., 2011).

The spatial organization of OM within the soil matrix, determining OM accessibility to decomposers and extracellular enzymes, can be identified as the main driver of SOC stability (Golchin et al., 1994; Kögel-Knabner et al., 2008; Schmidt et al., 2011). The association of silt and clay particles to form soil aggregates further protects OM from decomposition, because mechanisms of adsorption and occlusion operate simultaneously (Six et al., 2000; Dungait et al., 2012). Aggregates protect OM from microbial decomposition but, at the same time, stability of aggregate relies on OM turnover and microbial activity (Tisdall and Oades, 1982; Watts et al., 2005). Polysaccharides exuded from roots and produced by microbes adsorb to negatively charged clay surfaces through cation bridges and contribute to the stabilization of aggregates (Puget et al., 1998). An additional mechanism of physical SOC protection has been formulated, the "substrate-driven biological rate limitation" (Ekschmitt et al., 2005). The co-occurrence of water, air, substrate, enzyme and/or microbes controls SOM decomposition, meaning that a restricted connectivity between substrate and microorganisms, which commonly takes place in subsoils, leads to slow SOM decomposition (Dungait et al., 2012).

The responses of SOC stability to land-use and management changes can be investigated through physical fractionation methods, which allow to investigate the organization of OM within the soil matrix (Cambardella and Elliott, 1992; Six et al., 1998; Poeplau and Don, 2013). The concept of aggregate hierarchy, which recognizes the spatial organization of aggregates and their sequential formation (Tisdall and Oades, 1982), represented a milestone for the development of techniques to analyze SOM, which previously mainly relied on chemical procedures to isolate humic substances (Jarvis et al., 2012). Physical fractionation methods capture the effects of spatial organization on SOM dynamics and typically cause only limited alterations of the chemical SOM composition (Christensen, 1992; Olk and Gregorich, 2006). Physical fractions can be obtained through application of different disaggregating treatments (wet and dry sieving, slaking, ultrasonic vibration, shaking with glass beads) and separation by size and density (Six et al., 2002; Moni et al., 2012). In the strict sense, physical fractionation methods should separate SOM into primary organo-mineral complexes, and not into secondary organo-mineral complexes, the "aggregates" (Christensen, 1992). However, Chenu and Plante (2006) showed that "true" primary organo-mineral complexes as defined by Christensen (1996) do not correspond to reality but to conceptual entities, as particles <2 µm resembled very small microaggregates. The energy required to separate primary organo-mineral complexes would eventually lead to artifacts such as the breakdown of organic or mineral particles, and therefore to meaningless results for natural systems (Chenu and Plante, 2006). Aggregate isolation at different energy levels allows to clarify soil processes and agents that bind aggregates of different stabilities and at different spatial scales (Six and Paustian, 2014). The comparison of results obtained through the application of different energy levels to the same soil supported the aggregate hierarchy concept throughout the years, indicating that microaggregates (<250 µm) are sequentially formed within macroaggregates (>250 µm) (Angers et al., 1997; Six et al., 1998; Six et al., 2000). Microaggregates (<250 μm) rather than macroaggregates (>250 μm) protect SOC in the long-term, due to their greater stability against physical disturbances (Six et al., 2004).

The impact of forest colonization of abandoned grasslands on physical SOC fractions is unclear, as previous studies often focused on human-induced afforestation (Six et al., 2002; Poeplau and Don, 2013) or on the first phases of grassland abandonment before considerable forest expansion (Meyer et al., 2012b). After forest colonization, the formation of larger aggregates (e.g. macroaggregates) can be favored by the increasing abundance of coarse roots and fungi (Jackson et al., 1997; Zeller et al., 2000). However, aggregate stability may decrease due to the lower fine root input in forests compared with grasslands (Guo et al., 2007; Solly et al., 2013), the reduced quality (i.e higher lignin content and C/N ratio) of inputs (Hiltbrunner et al., 2013) and the lower soil temperature (Nosetto et al., 2005), which negatively affect microbial OM processing and thus the production of aggregate binding agents (Cotrufo et al., 2013). This can reduce OM incorporation into aggregates, leading to POM accumulation.

The chemical characterization of bulk SOC and physically separated fractions is needed to clarify the missing link between OM organization within the soil matrix and SOC chemical composition (Olk and Gregorich, 2006; Six and Paustian, 2014). Chemical extraction can isolate specific chemical OM components, as those relevant for aggregate stabilization. For example, the relative proportion of microbially to plant-derived carbohydrates can be used to explain changes in soil structure, considering the role of microbially-derived carbohydrates in aggregate stability (Cheshire, 1979; Roberson et al., 1995; Dungait et al., 2012). In forest-dominated successional stages, the decrease in easily decomposable OM inputs (e.g. carbohydrates) (Montane et al., 2010; Hiltbrunner et al., 2013) may cause a decrease in microbial processing and thus in carbohydrates of microbial origin (Cotrufo et al., 2013).

Thermal analysis has shown promising results for the characterization of SOC properties through analyses of bulk soil and physical soil fractions (Lopez-Capel et al., 2005; Leifeld et al., 2006; De la Rosa et al., 2008; Plante et al., 2011). Thermal analysis requires little sample preparation or pretreatments, and thus is an appealing alternative to conventional methods for SOC analysis (Plante et al., 2011). Differential scanning calorimetry (DSC) measures the heat release of a sample relative to a reference in function of temperature, which is generally raised to 600-1000°C (Fernández et al., 2011). The relative abundance of organic materials with different thermal stabilities can be determined through analysis of specific regions in the DSC thermogram, such as the first exothermic region (300-350°C), corresponding to the oxidation of thermally labile components (e.g. carbohydrates and aliphatics), and the second exothermic region (400-450°C), typically attributed to thermally resistant components (e.g. aromatics) (Dell'Abate et al., 2002). The determination of thermally labile and resistant components can therefore complement chemical analysis (Rovira et al., 2008; Creamer et al., 2012; Pérez-Cruzado et al., 2014) and be used as a proxy for biological SOC decomposability (Plante et al., 2011; Peltre et al., 2013; Leifeld and von Lützow, 2014). Measuring thermal properties of SOC may therefore help to unravel changes in SOC stability following forest succession on former grasslands, even though direct relationships between thermal attributes and the mechanisms involved into SOC stabilization have not been identified yet.

The combination of multiple approaches for SOC characterization such as physical fractionation, chemical and thermal analyses, is required to describe a range of stabilization mechanisms and to reach a holistic understanding of SOC changes (Olk and Gregorich, 2006). Therefore, this study integrated these complementary approaches to shed light on processes involved in SOC changes resulting from natural forest expansion on mountain grasslands.

Aim of the project

The aim of this PhD thesis was to investigate changes in SOC after forest expansion on mountain grasslands. Contrasting trends in soil organic carbon (SOC) stocks have been reported for mountainous regions following forest expansion on abandoned grasslands and its effects on SOC stability are largely unknown. To elucidate changes in SOC stocks and in its long-term stabilization, we aimed to characterize SOC using different approaches (physical, chemical and thermal analyses). Physical SOC protection is a major driver of SOC stability, therefore the investigation of physical soil fractions, such as aggregates and POM, can allow a better understanding of SOC stabilization processes. The responses of physical SOC protection to forest expansion on grasslands can be determined by variations in OM components that are easily decomposable, i.e. labile, that act as aggregate binding agents in soils.

In this PhD project, the following research questions were addressed:

- What are the responses of SOC stocks to forest expansion on abandoned grasslands? (PAPER I)
- How does physical SOC protection change following grassland abandonment and forest expansion? (PAPER II)
- Can physical SOC fractionation indicate emerging changes in the distribution of SOC stocks to various fractions? (PAPER III)
- How do labile soil C components respond to forest expansion on abandoned grasslands and can labile components explain changes in physical SOC protection? (PAPER IV)

Methods

Study area

The study area was located in Trentino, a mountainous region of the Southern Alps (Italy), where the forest area increased by 5% from 1973 to 1999 (Sitzia, 2009). Forest expansion in Trentino was investigated through the Neoboschi project, which showed that recently expanded forests have the following common features: (i) developed by natural regeneration; (ii) established on previous meadows or pastures; (iii) are located at an elevation of around 600-1200 m a.s.l.; and (iv) are located on south-facing gentle slopes (Sitzia, 2009). In order to investigate a forest expansion pattern typical for Trentino, our study area was selected among the areas identified by the Neoboschi project (Sitzia, 2009), ensuring that all common characteristics were included.

The study area is located in the municipality of Lavarone, with an elevation of around 1150 m a.s.l. (Fig. 1). The mean annual air temperature was 7.2°C and the mean annual precipitation was 1278 mm (1992-2011). The wettest months were October and November, while the driest month was February. The soil type is a Cambisol (IUSS, 2007), developed on calcareous parent material and with soil texture ranging from clay loam to clay.

We investigated the land-use history at the study area through comparison of orthorectified aerial photos (taken in 1954, 1973, 2006), historical land registers (1861, 1940) and via interviews of land owners and inhabitants. A land-use and management gradient was identified within a 4 ha area (Fig. 1), comprising: (i) managed grassland (manG), mown twice a year and manured once a year for at least the past 100 years; (ii) abandoned grassland (abanG), where a successional community of shrubs and Norway spruce (*Picea abies* (L.) Karst.) has developed in a grassland abandoned approximately 10 years ago; (iii) early-stage forest (earlyF), dominated by Norway spruce established in grassland abandoned around 1970; and (iv) old forest (oldF) composed mainly of European beech (*Fagus sylvatica* L.) and Norway spruce and already present in the historical land register of 1861. The successional stages manG, abanG and earlyF can be interpreted as phases of a LUC chronosequence, using a space-for-time substitution approach, with oldF as a reference for long-term forest land use. More information about management and floristic composition in each successional stage can be found in PAPER I.

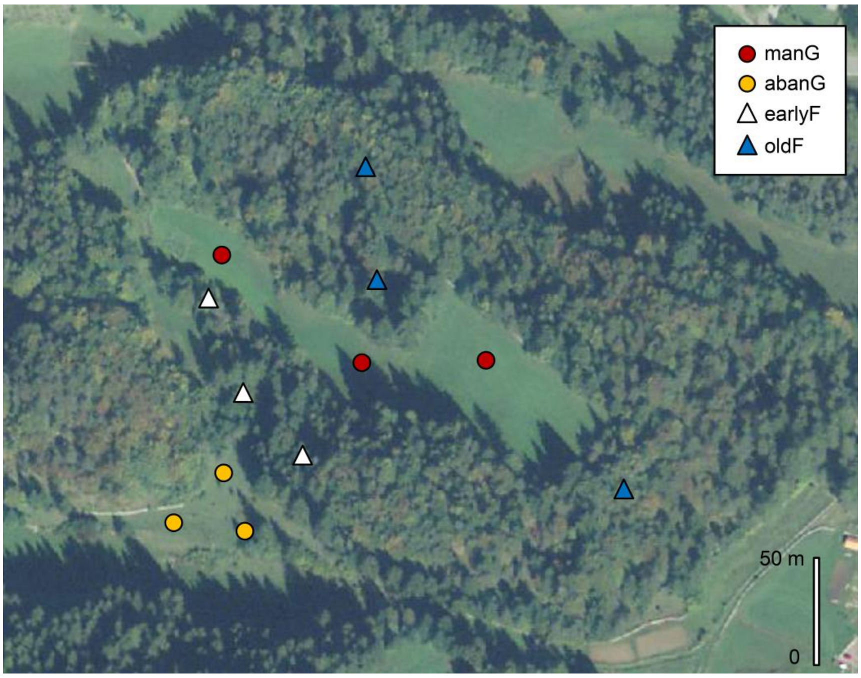

Fig. 1 Map of the study area, showing the plots investigated at the four successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Orthophoto from 2006 provided by Provincia Autonoma di Trento, Sistema Informativo Ambiente e Territorio

Soil sampling and processing

Soil was sampled in each successional stage within three circular plots (radius = 5 m) at eight sampling points. The investigated plots had similar topographic characteristics (elevation, low to medium slope, and south or south-east aspect), climatic conditions and the same soil type and parent material. Soils were sampled before deciduous trees shed their leaves (August–October, 2011 and 2012), corresponding to the annual minimum of organic layer mass (Vesterdal et al., 2002). The organic layers were sampled at earlyF and oldF within a 25x25 cm frame and were separated into LF (litter and fermentation) and H (humified) layers. At manG and abanG, the organic layers were collected from the same soil core used for mineral soil sampling (PAPER I). Following collection of the organic layers, the mineral soil was sampled with a soil corer to a depth of 30 cm, as this sampling depth allowed the investigation of the majority of SOC and N in the soil profile. The soil core was divided into four depth segments (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm), to detect if the effect of LUC on SOC was more pronounced at specific soil depths (PAPER I).

In the laboratory, the LF samples were separated into leaf and woody litter, while the H samples were separated into humified material and woody litter. The field-moist mineral soil samples were sieved at 8 mm, sorting out stones and roots, and let air-drying. A subsample <8 mm was taken out from each soil sample and pooled with samples collected at the same depth and plot in order to obtain a composite sample for aggregate size fractionation (PAPER II). The remaining soil was sieved at 2 mm, sorting out stones >2 mm and roots with length approximately greater than 2 cm. Organic layer materials, soil <2 mm and roots were dried at 55°C for 48 hours and weighed (Vesterdal et al., 2008). A subsample of each organic material and mineral soil was further dried at 105°C to constant weight for the determination of dry mass (PAPER I).

C and N stocks

The SOC and N stocks in the organic layers were calculated from the organic layer dry mass and OC and N concentrations, while mineral SOC and N stocks were calculated from the OC and N concentrations, soil bulk density, stone and root volume. Further details about determination of these soil properties can be found in PAPER I. Stocks were calculated according to an equivalent soil depth (ESD) and to an equivalent soil mass (ESM) approach (PAPER I). Compared to ESD, the ESM approach is not affected by variations in soil bulk density (Ellert and Bettany, 1995). For the ESM correction, we used as a reference the lightest average soil mass across the plots, according to the minimum ESM method by Lee et al. (2009). However, stocks were reported using an ESM approach only for the upper soil layers (0-5 and 5-10 cm), as in deeper soil layers the high stone content (> 20%) hampered the ESM correction, as pointed out by Schrumpf et al. (2011).

Tree aboveground biomass was estimated according to biomass equations developed for Trentino by Tabacchi et al. (2011). The C stock in tree biomass was calculated using a C concentration corresponding to the 50% of dry weight. Further information about the main forest stand features can be found in PAPER I.

Physical SOM fractionation

Mineral soil samples from the 0-5 cm, 5-10 cm and 10-20 cm layers were fractionated according to aggregate size fractionation and to size-density fractionation as shown in PAPER II and PAPER III. Aggregate size fractionation was performed according to Cambardella and Elliott (1993), through wet-sieving of soil <8 mm using three sieves (2000, 250, 53 μm). Size-density fractionation was performed according to a procedure modified from Magid et al. (2002) and Magid et al. (2010). The end-over-end shaking of soil samples with deionized water and glass beads was followed by wet-sieving at 50 μm. The fraction >50 μm was further separated through sodium polytungstate solution at a density of 1.6 g cm^{-3}. Three fractions were therefore collected: POM not occluded within stable aggregates (>50 µm, density <1.6 g cm^{-3}), stable aggregates (>50 µm, density >1.6 g cm^{-3}) and silt- and clay- sized fraction (<50 µm).

Carbohydrate analysis

Carbohydrate content and composition were assessed in bulk mineral soil samples (0-5 cm, 5-10 cm, 10-20 cm), size-density fractions (0-5 cm) and plant litter (roots, foliar and woody litter) as shown in PAPER IV. Samples were analyzed by two-step acid hydrolysis with sulfuric acid followed by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The major carbohydrate monomers in soil (glucose, xylose, arabinose, mannose, galactose, rhamnose and fucose) were quantified and the total carbohydrate content was calculated as the sum of individual carbohydrate monomers (Poirier et al., 2005). The mass ratio of microbially to plant-derived carbohydrates was calculated according to Murayama (1984) as: (rhamnose + fucose) / (arabinose + xylose), hereafter referred to as RF/AX ratio.

Thermal analysis

Thermal stability was measured in bulk mineral soil samples (0-5 cm, 5-10 cm, 10-20 cm) and in size-density fractions (0-5 cm) as shown in PAPER IV. Thermal stability was assessed through a differential scanning calorimeter (DSC), which measures the heat release of a sample relative to an empty aluminum pan, upon heating from room temperature to 600°C (heating rate of 10°C min^{-1}). Peak heights (normalized to initial organic carbon mass) of the first exotherm, second exotherm, their ratio and the peak temperatures of the first and second exotherm were used as thermal stability indices as shown in PAPER IV (Plante et al., 2009).

Results and Discussion

Changes in SOC stocks

Organic layers

The SOC stocks in the organic layers increased after forest development on abandoned grasslands, with an accumulation rate of 0.18 Mg C ha^{-1} yr^{-1} (Table 1; PAPER I). Our estimate falls in the lower range of organic layer C accumulation rates reported by studies investigating forest succession on grasslands in mountainous temperate regions (0.19-0.36 Mg C ha^{-1} yr^{-1}). This can be explained by the relatively high mean annual temperature in the study area compared with studies shown in Table 1. Temperatures positively influence both litter production and decomposition, but the relative increase in decomposition appeared to be greater than the increase in production (Kirschbaum, 2000). However, accumulation rates similar to ours were reported by Gamper et al. (2007), even though their site had much lower mean annual temperature.

Table 1 Organic layer C stock changes following forest succession on grasslands in mountainous temperate regions

Study	**Region**	**Time since abandonment (yrs)**	**Elevation (m a.s.l.)**	**MAT[a] (°C)**	**Former land use**	**Tree species**	**Organic layers (Mg C ha^{-1} yr^{-1})**
Thuille et al. (2000)	Italy	62	1130-1390	n.a.	meadow	spruce	0.36
Thuille and Schulze (2006)	Italy Germany	112	1700-1775	4.1	meadow	spruce	0.24
Gamper et al. (2007)	Italy	30	1770	3.6	meadow	coniferous	0.19
Montane et al. (2007)	Spain	25	1650-2100	3-7	pasture	coniferous	0.33
PAPER I	***Italy***	***42***	***1150***	***7.2***	***meadow***	***spruce***	***0.18***

[a]MAT = mean annual temperature

The C accumulation rates within the organic layers can be affected by tree species composition (Vesterdal et al., 2008). Although Norway spruce was the dominant tree species, deciduous species as *Corylus avellana* L. were present in relevant proportions in earlyF, and this may have influenced overall organic layer accumulation rates. As shown in a recent meta-analysis of afforestation effects on SOC in Northern Europe (Bárcena et al., 2014), mixed or deciduous forests tend to accumulate C in the organic layers at lower rates than conifer forests (0.10 vs 0.35

Mg ha^{-1} yr^{-1}), likely due to differences in litter quality and in microclimate that influence decomposition rates (Vesterdal et al., 2012).

The organic layer C stocks at oldF (27 Mg C ha^{-1}) are in line with organic layer C stocks of managed spruce- and beech-dominated forests in the Bavarian Alps (Christophel et al., 2013) and of mature spruce forest in the Swiss subalpine region (Hiltbrunner et al., 2013), which stored on average 25 Mg C ha^{-1}. However, the time needed to reach a steady-state condition, i.e. C inputs equal outputs, may vary among studies. It is likely that organic layer C stocks at earlyF have not reached a steady state yet, because much more C was stored in the organic layers under oldF than earlyF. Around additional 100 years would be needed to attain a C stock level equivalent to that in oldF, assuming a constant C accumulation rate of 0.18 Mg C ha^{-1}. Similarly, in a review by Poeplau et al. (2011), organic layer C stocks increased linearly for over 200 years after conversion of cropland/grassland to forest. On the contrary, Hiltbrunner et al. (2013) reported that 45 years were enough to reach a steady-state condition for organic layer C stocks following pasture afforestation, and Thuille and Schulze (2006) showed that organic layers approached a steady state after 60 years of spruce development, which was attributed to a relatively rapid re-establishment of earthworm populations during forest development (Bernier and Ponge, 1994).

Mineral soil

Our study revealed that oldF had significantly lower SOC concentrations than manG and abanG in the 0-5 cm layer and that the SOC stocks in the upper soil layers (0-5 cm and 5-10 cm) gradually decreased from manG to oldF (PAPER I). Similarly to the upper soil layers, we found that mineral SOC stocks in the 0-30 cm layer decreased from grasslands to forest successional stages, which had SOC stocks 40-50% lower than grassland successional stages (PAPER I). Therefore, our results showed that forest expansion on grassland caused a significant and progressive decrease in mineral SOC stocks.

Studies that investigated forest expansion on grasslands reported contrasting directions and magnitude for changes in mineral SOC stocks following grassland abandonment, ranging from -1.6 to +0.5 Mg C ha^{-1} yr^{-1} (Table 2). Time since abandonment could have affected the rates of mineral SOC stock change observed in the different studies, leading to SOC accumulation with increasing age of the abandoned grassland (Fig. 2), in agreement with Montane et al. (2007) who showed that SOC stocks were positively and significantly related to the age of the abandoned grasslands.

Therefore, the time scale of the study may have an impact on rates and overall SOC stock changes following grassland abandonment.

Table 2 Mineral SOC stock changes following grassland abandonment and forest expansion in mountainous temperate regions

						abanG		earlyF		oldF
Study	**Region**	**Elevation (m a.s.l.)**	**Former land use**	**Tree species**	**Soil depth (cm)**	**Time (yrs)**	**ΔSOC[1] (Mg C ha^{-1} yr^{-1})**	**Time (yrs)**	**ΔSOC[2] (Mg C ha^{-1} yr^{-1})**	**ΔSOC[3] (%)**
Thuille et al. (2000)	Italy	1130-1390	meadow	spruce	0-15	3	-1.63	62	-0.30	-60
Thuille and Schulze (2006)	Italy Germany	1000-1775	meadow	spruce	0-50	n.a.	n.a.	112	-0.18	-10/-40
Montane et al. (2007)	Spain	1650-2100	pasture	conif. shrubs	0-30	25[4]	+0.51	n.a.	n.a.	n.a.
Risch et al. (2008)	Switzerland	1350-3170	pasture	conif.	0-20	n.a.	n.a.	n.a.	n.a.	-35
Alberti et al. (2008)	Italy	600	meadow	decid.	0-30	n.a.	n.a.	75	-0.66	n.a.
Alberti et al. (2011)	Italy	600-1000	meadow	decid.	0-30	n.a.	n.a.	23/67	-0.51/ -1.14	n.a.
Meyer et al. (2012)	Austria Italy	1790-2000	meadow, pasture	conif. shrubs	0-10	10-25[4]	+0.14/ +0.54	n.a.	n.a.	n.a.
PAPER I	***Italy***	***1150***	***meadow***	***spruce***	***0-30***	***10***	***-1.32***	***42***	***-0.83***	***-50***

[1] (SOC abanG –SOC manG)/(years since abandonment)
[2] (SOC earlyF –SOC manG)/(years since abandonment)
[3] (SOC oldF –SOC manG)*100/(SOC manG)
[4] Grassland was invaded by shrubs (height 0.2-1 m) rather than by trees and thus included in abanG although a relatively long time since abandonment

Different climatic conditions can also be a reason for the inconsistent SOC changes reported following grassland abandonment. After woody plant invasion on grasslands, a negative relationship was shown by Jackson et al. (2002) between precipitation and SOC stock changes. In fact, in high rainfall areas, grassland abandonment may cause SOC losses, and a possible threshold for mean annual precipitation can be identified around 900-1000 mm also for mountainous areas (Table 2), with drier sites increasing and wetter sites losing C (Fig. 2). Similarly, Guo and Gifford (2002) showed for lowland sites that pasture-to-forest conversions negatively affected SOC stocks in high rainfall areas (>1200 mm), whereas the same land conversion had little effect on SOC stocks in areas with rainfall <1200 mm. According to Guo and Gifford (2002), woody plants may be less effective than grasses at storing C in some environments. In a global dataset, the relationship between SOC and mean annual precipitation had a slope 2.6 times higher for grasslands than for woodlands, with the latter having 43% less SOC than grasslands at a precipitation level of 1000 mm (Jackson et al., 2002). Moreover, N leaching can occur especially in the initial phases of grassland abandonment, thus causing a subsequent reduction in SOC stocks (Kirschbaum et al., 2008). In our study, the relatively short time since abandonment (10 years) and the high amount of rainfall (around 1300 mm) may explain the observed decrease in SOC stocks following grassland abandonment. Considering the low number of studies in mountainous regions, however, it is not possible to isolate the effects of time and climate on SOC stocks, as both factors could have contributed to the observed trends.

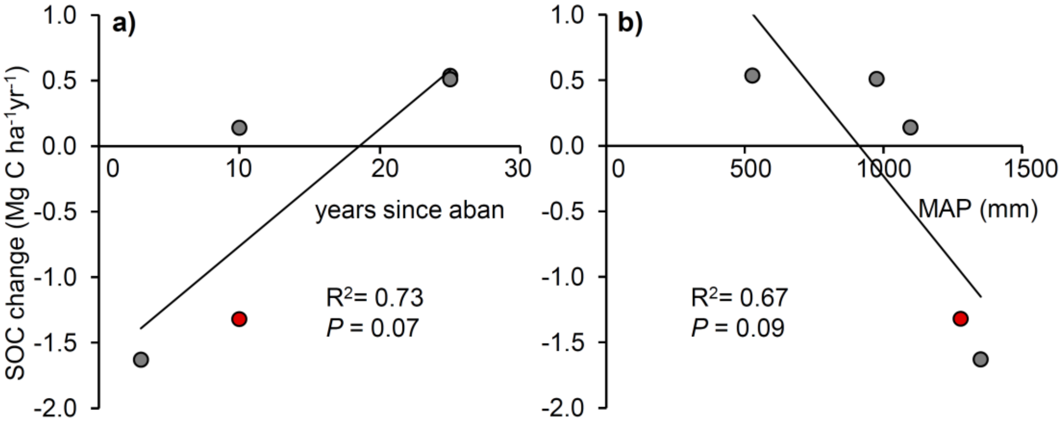

Fig. 2 Relationship between annual mineral SOC stock changes and: (**a**) years since abandonment (aban); and (**b**) mean annual precipitation (MAP) observed in studies dealing with grassland abandonment in mountainous temperate regions (Table 2). Our study (PAPER I) is shown as a red circle

Annual mineral SOC stock changes from abandonment of grassland to early-stage forest ranged from -0.2 to -1.1 Mg C ha^{-1} yr^{-1} (Table 2). A decrease in the loss rate of mineral SOC with increasing time since abandonment is shown in Fig. 3. Early stages of forest succession (40-50 years since abandonment) showed large annual SOC losses, as in PAPER 1, whereas later stages of forest succession showed small annual SOC changes, as they may have approached a steady-state C level (annual SOC change ~ 0). Post and Kwon (2000) previously reported slowing rates of accumulation or loss during forest establishment on agricultural lands as a new SOC steady-state condition was established. More than 100 years could be necessary to reach a new SOC stock level following forest expansion on mountain grasslands (Thuille and Schulze, 2006). In temperate regions, Poeplau et al. (2011) showed that more than 150 years were necessary to reach a SOC steady state following grassland afforestation, in agreement with natural succession in mountainous areas (Fig. 3). The transition period before reaching a SOC stock steady state may have such a long duration because OM turnover is slower in cold than in warm regions (Trumbore, 1993), and thus the balance between OM input and output from soils takes a longer time to establish (Olson, 1963).

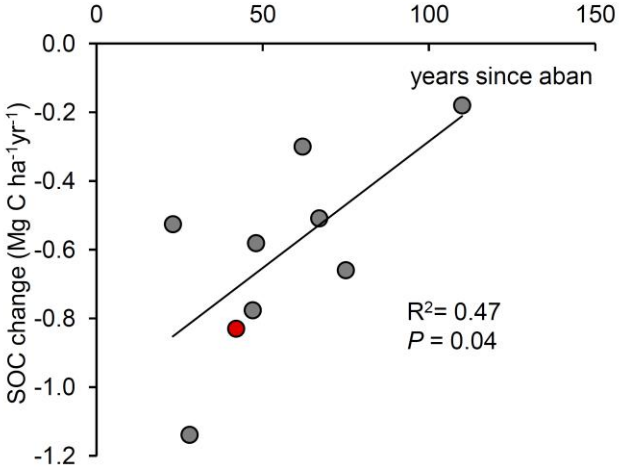

Fig. 3 Relationship between annual mineral SOC stocks changes and years since abandonment (aban) observed in studies dealing with forest expansion on grasslands in mountainous temperate regions (Table 2). Our study (PAPER I) is shown as a red circle

The shift from grassland to forest was reported to have a marginal impact on mineral SOC stocks by reviews of Guo and Gifford (2002), Laganiere et al. (2010) and Poeplau et al. (2011). Also in Northern Europe, a small, non-significant effect of grassland afforestation on mineral SOC stocks was reported by Bárcena et al. (2014). However, these studies dealt with human-induced afforestation, which is specifically carried out to maximize biomass production and then, indirectly, C accumulation. The naturally established forest stands are generally less productive, especially in the first phases of establishment, and accumulate less C than plantations (Anderson et al., 2006; Tremblay and Ouimet, 2013). This may be the reason for a more marked decrease in mineral SOC stocks in natural succession than in afforestation of grasslands. Nevertheless, site preparation before tree planting can enhance SOC mineralization, which can lead to SOC losses in the first years since afforestation (Jandl et al., 2007; Don et al., 2009).

A sampling depth of 30 cm was the most common across studies in mountainous regions (Table 2), likely due to the shallow parent material and the presence of stones and boulders. We also chose to sample the mineral soil from 0 to 30 cm, as this corresponded to the average soil depth in our study area. Thuille and Schulze (2006) sampled the mineral soil layers to 50 cm, and they did not reveal different patterns in SOC changes between upper and deeper soil layers. Poeplau and Don (2013) showed that changes in deeper layers (below 30 cm) SOC stocks were in the same direction as SOC changes in the upper layers, and reported slight to significant SOC losses in most of their sites after grassland afforestation.

Under similar long-term continuity, the mineral SOC stocks were 10 to 60% lower under forest than under managed grassland in mountainous regions (Table 2). Our study showed a significant SOC difference (-50%) between manG and oldF, which is in line other studies in mountainous regions (Table 2). Wiesmeier et al. (2014) also reported that mineral SOC stocks were 39% lower in forest than in grassland sites located across a broad range of soil types in southeast Germany.

The estimate of SOC stock changes provided by our study is aligned with studies comparing grassland and forest sites in similar mountainous environments. Although a relatively small area was investigated in this project, the comparison with other studies confirmed that our study area was reasonably representative of forest expansion in mountainous environments.

The lower mineral SOC content found under forest compared with grassland successional stages can be attributed to differences in the ecology of these land uses. Forests tend to allocate more C inputs aboveground through litterfall, while grasslands allocate more C inputs belowground through roots and exudates (Jobbagy and Jackson, 2000; Guo et al., 2008). The lower mass and slower turnover of fine roots found under forest compared with grassland sites (Guo et al., 2007; Solly et al., 2013) can be partly responsible for the lower mineral SOC stocks found in forest than in grassland sites. Moreover, the application of manure could have increased the grassland SOC content directly through the input of additional C or indirectly through the enhancement of grassland productivity (Conant et al., 2001), thus affecting the SOC stocks observed in grassland and forest successional stages.

Ecosystem OC stocks

The decrease in mineral SOC stocks following forest expansion on abandoned grasslands was compensated by C accumulation in the tree biomass and organic layers approximately 26 years after grassland abandonment. However, the C accumulation within the organic layers could not fully offset the mineral SOC stock difference between forest and grassland successional stages (PAPER I). Total SOC stocks were, on average, lower in earlyF and oldF than in manG. This suggest that the organic layer build-up may not always compensate the lower mineral SOC stocks in forest successional stages, in agreement with Poeplau and Don (2013) and Hiltbrunner et al. (2013). Moreover, the difference in total SOC stocks between manG and oldF (-21%), although not significant, is in line with results reported by Wiesmeier et al. (2014), who found that total SOC stocks were 26% lower in forest than in grassland sites located within low to high-elevation areas in southeast Germany.

Development of tree biomass contributed to increasing ecosystem OC stocks in forest successional stages (Fig. 4). Especially the long-term forest land use, in fact, stored more C in tree biomass and organic layers, but C accumulated in these pools is considered more susceptible against external disturbances, such as management, harvesting and environmental modifications compared with C stored in the mineral soil (Gaudinski et al., 2000; Vesterdal et al., 2002). While organic layers and tree biomass accounted for the majority of ecosystem OC stocks in forest successional stages, the contribution of mineral soil to overall ecosystem OC stocks markedly decreased from manG to oldF, as shown in Fig. 4.

In brief, forest successional stages positively contributed to atmospheric CO_2 removal through build-up of tree biomass and organic layers, but the C stored in these pools may be less stable against management and environmental changes than the C stored in the mineral soil.

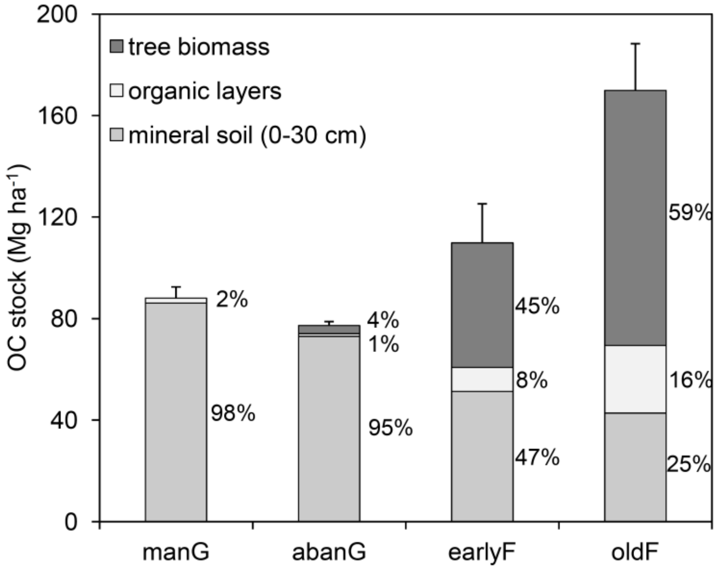

Fig. 4 Ecosystem OC stocks at the four successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Mineral SOC stocks are calculated using an equivalent soil depth approach and considering the total stone content. Error bars represent the standard error of the mean (*n* = 3). Values in percentage (%) indicate the contribution of different pools to ecosystem OC stocks

Changes in physical SOC fractions

Changes in aggregate size and stability

The use of two fractionation procedures (aggregate size fractionation and size-density fractionation), separating aggregates at different physical disturbance levels, allowed to gain a integrated understanding of aggregate size and stability changes following forest expansion on grasslands (PAPER II and III). The dimension of aggregates in the 0-5 cm layer tended to increase following conversion from grassland to forest, even though no significant difference was found among successional stages in the SOC allocation to aggregate size fractions (Fig. 5). The results obtained through size-density fractionation showed a decrease in the relative SOC allocation to stable aggregates following forest expansion on grassland (from 81 to 59% in the 0-5 cm layer; Fig. 5). Our study therefore revealed that aggregates tended to be larger but less stable in forest-dominated than in grassland successional stages. Differences in aggregate size fractions and stable aggregates from size-density fractionation were more evident in upper soil layers (e.g. 0-5 cm layer), in agreement with findings that upper soil layers are affected to a greater extent by LUC than deeper layers (Poeplau and Don, 2013).

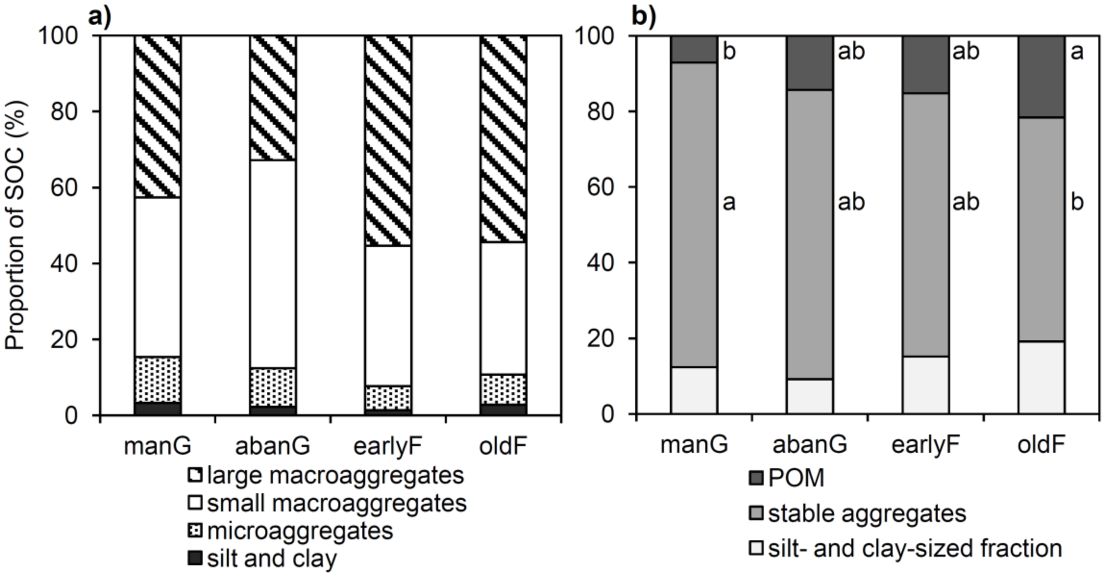

Fig. 5 Proportion of SOC in: (**a**) aggregate size fractions; and (**b**) size-density fractions in the 0-5 cm layer at the four successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Within each size-density fraction and depth, different letters indicate significant differences based on multiple comparisons following Kruskal-Wallis, with $P < 0.05$. If no letters are present, no significant differences were observed among successional stages

Only few studies explored changes in aggregate size and stability following natural forest expansion on grasslands (Table 3). Table 3 also includes results from studies focusing on human-induced afforestation of grasslands or on the comparisons of long-term grassland and forest land uses, to elucidate if grassland and forest land uses were characterized by intrinsic differences in aggregate size and stability.

The aggregate size slightly decreased or remained unchanged in the first phases of grassland abandonment in mountainous regions (Table 3), as shown by Meyer et al. (2012b) and in PAPER II. Our study did not show a significant change in mean weight diameter (MWD) or in the SOC allocation to large macroaggregates between manG and abanG (Fig. 5), suggesting that aggregate size remained almost unvaried following grassland abandonment. When grasslands were colonized by woody plants, the mass proportion of macroaggregates increased, as shown by Liao et al. (2006). Our study also indicated higher MWD in forest than in grassland successional stages (PAPER II). However, John et al. (2005) found no difference in MWD between forest and grassland sites.

The SOC allocation to stable aggregates slightly, but not significantly, increased from manG to abanG in the 0-20 cm layer (PAPER II). A significant decrease was registered from grassland to forest successional stages (PAPER II) and for grassland afforestation (Poeplau and Don, 2013). Similarly, Wiesmeier et al. (2014) reported lower SOC allocation to stable aggregates in forest than in grassland land uses. Soil texture may influence changes in aggregate stability following LUC, however the results of our study, carried out on fine-textured soils, are in line with studies comparing grassland and forest sites across a broad range of soil types and textures (Poeplau and Don, 2013; Wiesmeier et al., 2014).

Table 3 Changes in aggregate size, C allocation to stable aggregates and POM in grassland-to-forest conversions

Study	**Region**	**Elevation (m a.s.l.)**	**Former land use**	**Woody species**	**Soil depth (cm)**	**Time (yrs)**	**Aggregate size**	**Δaggregate C allocation[1] (%)**	**ΔPOM C allocation[1] (%)**
Shrub and tree colonization of abandoned grasslands									
Liao et al. (2006)	Texas (USA)	75-90	grassland	legume shrubs	0-30	10-130	+ macro	n.a.	+75/ +125
Clark et al. (2012)	New England (USA)	253–612	meadow, pasture	decid.	0-20	25-40	n.a.	n.a.	-16/-40
Meyer et al. (2012b)	Austria Italy	1790-2000	meadow, pasture	conif. shrubs	0-10	10-25	=/ -	n.a.	+41/+200
PAPER II	***Italy***	***1150***	***meadow***	***spruce***	***0-20***	***10-42***	***=/ +***	***+10/-4***	***+72/ +121***
Grassland afforestation									
Poeplau and Don (2013)	Europe	231-1792	grassland	conif. decid.	0-30	22-75	n.a.	-19	+72
Comparisons of long-term grassland and forest land uses									
John et al. (2005)	Germany	360	meadow	spruce	0-30	80	=	n.a.	+271
Wiesmeier et al. (2014)	Germany	107-2962	grassland	conif. decid	A horizon	n.a.	n.a.	-10	+223
PAPER II	***Italy***	***1150***	***meadow***	***spruce***	***0-20***	***>150***	***+***	***-18***	***+243***

[1] (SOC% shrub or forest – SOC% manG)*100/(SOC% manG)

Changes in C allocation to POM fraction

Our study showed that the SOC allocation to POM gradually increased from manG to oldF in the 0-5 cm (Fig. 5), 5-10 cm and 10-20 cm layers, with intermediate values in abanG and earlyF (PAPER II).

The SOC allocation to POM increased following grassland abandonment (Meyer et al., 2012; PAPER II), shrub and tree establishment on grasslands (Liao et al., 2006; PAPER II), grassland afforestation (Poeplau and Don, 2013), and it was higher in forest than in grassland land uses (John et al., 2005; Wiesmeier et al., 2014; PAPER II). The increase in POM C was evident not only for fine-textured soil (PAPER II), but also for coarse-textured soil (Liao et al., 2006) and for studies carried out in a broad range of soil types and textures (Poeplau and Don, 2013; Wiesmeier et al., 2014). Among studies shown in Table 3, only Clark et al. (2012) observed that the proportion of POM C decreased following forest establishment on abandoned grasslands. This was probably due to tillage operations in the managed grasslands, which lead to the disruption of soil aggregates and thus to higher abundance of unprotected POM, or to the prevalent tree species composition, as litter derived from deciduous tree species may have favored OM decomposition and POM incorporation into mineral associations.

Contribution of size-density fractions to total SOC stocks

The decrease in mineral SOC stock following forest expansion on former grasslands mainly took place in stable aggregates, while POM-C tended to increase from manG to oldF (+3.8 Mg C ha-^{1} in the 0-20 cm layer; Fig. 6). The decreased SOC allocation to stable aggregates and increased POM-C allocation (Fig. 6) suggest that the physical protection of mineral SOC declined in the succession from grassland to forest. When ecosystems are disturbed, POM-C often undergo rapid losses, as observed for conversions from native ecosystems to agricultural land uses (Cambardella and Elliott, 1992; Dalal et al., 2005), but in some cases POM can retain substantial amounts of C following disturbances (Gregorich et al., 2006). Moreover, physical protection of SOC within the soil layers further decreased due to the increased organic layer contribution to total SOC stocks (Fig. 6; PAPER I), as organic layers respond rapidly to environmental and management modifications (Currie, 1999). The POM and organic layers can be considered as labile (or active) C pools according to Wiesmeier et al. (2014). Therefore, forest successional stages are characterized by higher amounts (34 Mg C ha^{-1}) and contributions (47% in oldF) of labile C to total SOC stocks than grasslands (5 Mg C ha^{-1}, 8% of total stocks in manG, Fig. 6). Similarly, Wiesmeier et al. (2014) reported that the labile pools accounted for 10% of total SOC stocks in grasslands and almost 40% of total SOC stocks in forest land uses. The increased contribution of labile C therefore suggest that SOC stocks can become more susceptible to management and environmental changes.

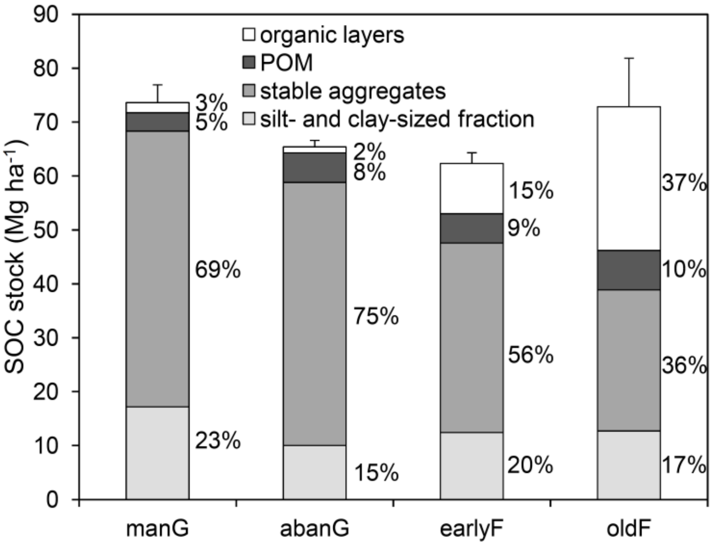

Fig. 6 SOC stocks in size-density fractions within the mineral soil (0–20 cm) and in organic layers at the four successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Error bars represent the standard error of the mean of total SOC stocks (n = 3). Values in percentage (%) indicate the contribution of fractions and organic layers to total SOC stocks

Mechanisms behind changes in soil aggregation and POM

Differences in the ecology of grasslands vs. forests can affect aggregate size and stability (Fig. 7). Differences in root systems and associated microbial communities may have been the major drivers of changes in aggregate size, which increased following forest succession on grasslands (PAPER II; Liao et al., 2006). Trees generally have larger root diameter than grasses (Jackson et al., 1997) and greater fungal to bacterial ratios (Macdonald et al., 2009), which are known to increase physical enmeshment of soil aggregates (Tisdall, 1991) and thus may have increased aggregate size following forest succession. On the other hand, lower fine root input (Guo et al., 2007; Solly et al., 2013) and lower abundance of arbuscular mycorrhizal fungi (i.e. AMF) compared to ectomycorrhizal fungi (i.e. EcMF) (Macdonald et al., 2009) may have contributed to lower aggregate stability in forests than in grasslands (Poeplau and Don, 2013; Wiesmeier et al., 2014; PAPER II), considering their enhancing effect on aggregate stabilization (Jastrow et al., 1998; Bedini et al., 2009). Microbial activity indirectly promotes aggregate stabilization, through production of microbially-derived components that enhance the formation of stable aggregates (Cotrufo et al., 2013). Lower litter quality (e.g. low N and high lignin) (Hiltbrunner et al., 2013), the cessation of manure application (Aoyama et al., 2000), and reduced soil temperatures (Nosetto et al., 2005) may have decreased microbial activity in forests compared with grasslands, as shown by

Ross et al. (2002), with a resulting decrease in the production of microbially-derived compounds and thus in aggregate stability. Similarly, the slow-down of microbial OM decomposition processes could have caused the accumulation of POM not occluded within stable aggregates following forest establishment on grasslands (Liao et al., 2006; Poeplau and Don, 2013; PAPER II). Moreover, the lower root litter quality and the prevalence of coarse roots vs. fine roots in forests (Silver and Miya, 2001; Guo et al., 2008) could have contributed further to increase POM accumulation in forest than in grassland successional stages.

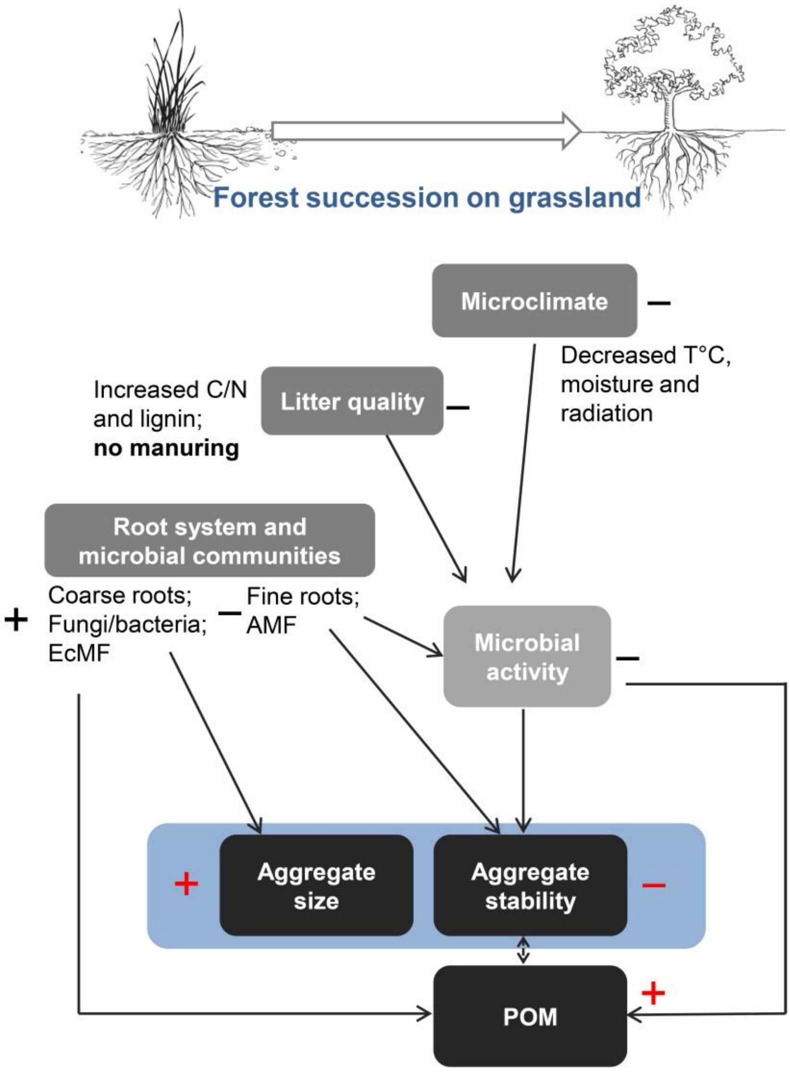

Fig. 7 Mechanisms behind changes in soil aggregation and in particulate organic matter (POM) following forest succession on grassland

Changes in chemically and thermally labile soil C

The observed decrease in physical SOC protection (PAPER II) suggested that the OM components involved into aggregate stabilization, such as microbially-derived carbohydrates, would decrease following forest expansion on grasslands. A lower proportion of labile components (i.e. easily decomposable, such as carbohydrates) in OM inputs, together with a higher proportion of components with greater stability against decomposition (i.e. lignin) (Montane et al., 2010; Hiltbrunner et al., 2013) may reduce the production of microbially-derived compounds (Cotrufo et al., 2013), therefore causing a decrease in aggregate stability following forest colonization of mountain grasslands.

Changes in chemically and thermally labile C in bulk soil and size-density fractions

Carbohydrate analysis (PAPER IV) indicated greater proportions of labile soil C (carbohydrate C/SOC) in abanG than in manG and oldF within the bulk soil and mineral-associated OM fractions (stable aggregates and silt- and clay-sized fraction) in the 0-5 cm layer (Fig. 8a).

The greater share of carbohydrate C in abanG than in manG may have been caused by a slower transformation of litter inputs, for example due to cessation of manure application (Knorr et al., 2005), rather than by differences in the content of carbohydrates C in roots and foliar litter (PAPER IV). The trend toward higher carbohydrate C in abanG than in oldF, however, may have mainly resulted from differences in the quality of litter input, as shown in PAPER IV and in former studies (Sanger et al., 1997; Montane et al., 2010). On the contrary, carbohydrate C did not differ among manG and forest successional stages in bulk soil and mineral-associated OM fractions although plant materials in manG were richer in carbohydrates. The carbohydrate-rich litter in manG, together with the more favorable microclimatic conditions (Nosetto et al., 2005; Hiltbrunner et al., 2013) and the annual input of manure, may cause faster decomposition of C inputs (Berg and McClaugherty, 2003; Knorr et al., 2005; Hiltbrunner et al., 2013). This resulted in similar shares of carbohydrate C in soil (Fig. 8a; PAPER IV). Similarly, Creamer et al. (2012) reported no difference in carbohydrate C between grassland and woody successional stages for a site in Southern Texas, while Pérez-Cruzado et al. (2014) observed a decrease in chemically labile soil C (O-alkyl and alkyl compounds) following pasture afforestation in Spain.

The POM fraction showed more pronounced differences in carbohydrate C among successional stages than bulk soil and other fractions. Higher content of carbohydrate C in POM in grassland than in forest-dominated successional stages may reflect differences in litter quality and in the localization of OM decomposition processes in the soil layers, as carbohydrate consumption during spruce litter decomposition in the organic layers was previously reported by Helfrich et al. (2006).

Thermal analysis indicated higher abundance of thermally labile components (peak height 1) in abanG than in manG and oldF within the bulk soil, similarly to carbohydrate analysis. This is in agreement with Leifeld et al. (2006) and Pérez-Cruzado et al. (2014), who showed the relevance of similar thermal analysis parameters for characterizing changes in labile components within the bulk soil. However, no significant difference in thermally labile components was shown within physical soil fractions. Considering that thermal properties depend not only on chemical characteristics but also on the degree of polymerization, spatial arrangement and mineral associations (Leifeld, 2008; Plante et al., 2009), it is not possible to give a straightforward and unequivocal interpretation of the thermal behavior of bulk soil and fractions.

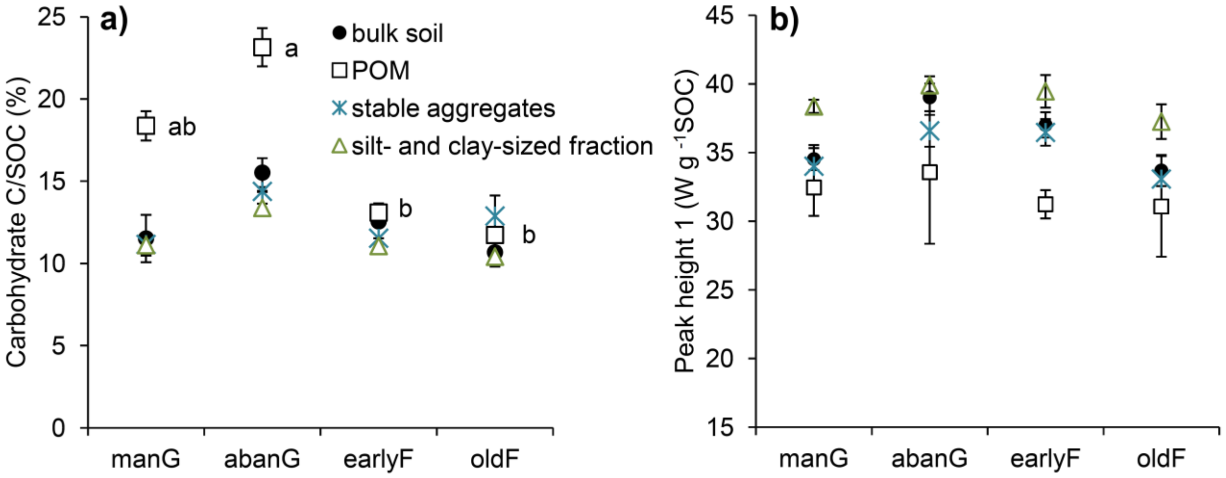

Fig. 8 Proportion of SOC stored as: (**a**) carbohydrate C; and (**b**) peak heights of the first exotherm (peak height 1) within bulk soil and size-density fractions in the 0-5 cm layer at the successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Error bars represent the standard error of the mean ($n = 3$). Within each fraction, different letters indicate significant differences based on multiple comparisons after Kruskal-Wallis, with $P < 0.05$. If no letters are present, no significant differences were observed among successional stages

Carbohydrate origin in bulk soil and fractions

Analysis of carbohydrate monomers revealed that ratios of microbially to plant-derived carbohydrates decreased from grassland to forest successional stages within bulk soil and mineral associated fractions (Fig. 9; PAPER IV). The ratio of microbially to plant-derived carbohydrates

(RF/AX) showed a gradually decreasing trend from manG to oldF in stable aggregates and a similar pattern was also detected in bulk soil and silt and clay (Fig. 9). The prevalent microbial origin of rhamnose and fucose was confirmed by their small proportions within plant litter (PAPER IV), in agreement with Schädel et al. (2010).

Galactose and mannose are also considered to derive mainly from microbial synthesis in soil (Oades, 1984; Guggenberger et al., 1995), but hemicellulose of conifers can contain high proportions of these sugars as well (Kögel-Knabner, 2002; Schädel et al., 2010; Prietzel et al., 2013). The higher abundance of galactose in manG than in oldF can be explained by its higher microbial synthesis and/or preservation in mineral-associated fractions, which compensated for the initially lower galactose proportions in grass litter (PAPER IV). In the case of mannose, however, microbial production was probably not sufficient to modify the carbohydrate signature of litter input to bulk soil (PAPER IV).

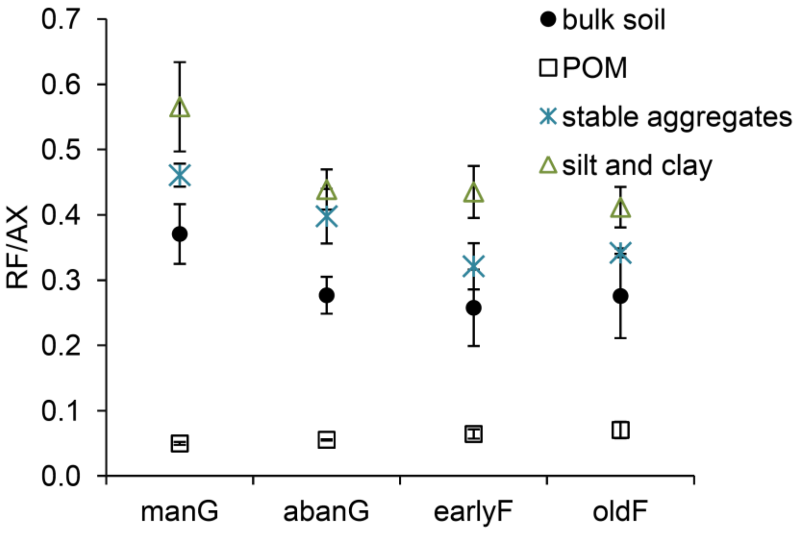

Fig. 9 Ratio of microbially to plant-derived sugars, RF/AX = (rhamnose + fucose)/(arabinose + xylose) within bulk soil and fractions in the 0-5 cm layer at the successional stages (manG: managed grassland; abanG: abandoned grassland; earlyF: early-stage forest; oldF: old forest). Error bars represent the standard error of the mean (n = 3). If no letters are present, no significant differences were observed among successional stages within each fraction based on multiple comparisons after Kruskal-Wallis, with $P < 0.05$

We interpreted the decreasing proportions of microbially to plant derived carbohydrates from manG to oldF as the result of decreased microbial OM processing following forest succession on grassland. Additionally, the prevalently plant-derived carbohydrates (e.g xylose and glucose) decreased from plant litter to bulk soil, and also from POM to stable aggregates and silt and clay, while the microbially-derived carbohydrates (e.g rhamnose, fucose) followed an opposite trend, likely due to their microbial synthesis and stabilization in the mineral matrix (Kiem and Kögel-Knabner, 2003; Rumpel et al., 2010). These patterns were more evident in grassland than in forest successional stages, thus supporting the hypothesis of greater microbial processing in grasslands. This is also consistent with previous studies that showed lower soil microbial biomass and activity in afforested than in pasture sites (Ross et al., 2002; Macdonald et al., 2009).

Former studies have mainly explored human-induced afforestation or long-term land uses rather than natural forest expansion, showing inconsistent patterns. Higher proportions of microbially to plant-derived carbohydrates were reported in forest compared with grassland by Guggenberger et al. (1994), while Sanger et al. (1997) showed that SOM under spruce contained lower proportions of microbially-derived carbohydrates compared with a pasture.

Linking labile carbon to aggregate stability

Our study showed that carbohydrates, especially those originated from microbial synthesis (i.e. rhamnose and galactose) were significantly and positively related to C allocation to stable aggregates obtained from size-density fractionation (PAPER IV). Carbohydrates of microbial origin are more likely to be stabilized in the long-term by mineral interactions or occlusion within aggregates than carbohydrates of plant origin (Kiem and Kögel-Knabner, 2003; Rumpel et al., 2010). Moreover, microbially-derived carbohydrates actively enhance aggregate stabilization, acting as bridges between soil particles (Dungait et al., 2012).

Our study also revealed a positive relationship between the ratio of thermally labile vs. resistant components and the C allocation to stable aggregates, confirming the link found between carbohydrates and stable aggregates. Therefore chemically and thermally labile SOM components seemed to be involved in the formation of stable SOM, in agreement with the conceptual framework proposed by Cotrufo et al. (2013). Our study provides evidence for their hypothesis that labile OM inputs, although easily decomposable, are the dominant source of microbial products and paradoxically represent the major precursors of stable SOM.

Fractionation procedures to elucidate changes in SOC stability

The separation of stable from labile soil C fractions is crucial to determine soil C stabilization mechanisms and soil C stability responses to LUC. Theoretically, the soil C fractions should be distinguished on the basis of their turnover time in soil, which ranges from months-years (referred to as labile or active C) to several centuries (referred to as stable or passive C) (Barré et al., 2010). However, SOC stability depends on complex interactions between intrinsic characteristics of organic matter and its biotic and abiotic environment (Schmidt et al., 2011), and thus the separation of stable and labile C pools remains a challenge (von Lützow et al., 2007; Zimmermann et al., 2007; Bruun et al., 2008). Therefore, throughout our study, we aimed to separate soil C fractions representative of SOM processes and sensitive to LUC (PAPER II, III, IV) using a combination of different approaches (physical, chemical and thermal methods) rather than to separate fractions into "pools" with homogenous turnover times, as these may eventually represent "*ghostly artefacts*", as discussed by Andrén et al. (2008).

Physical procedures that separate soil aggregates are based on the assumption that physical protection within the soil structure determines SOC accessibility to decomposers and enzymes and thus these procedures can isolate fractions more directly related to SOC turnover than those separated by chemical procedures (Kögel-Knabner et al., 2008; Schmidt et al., 2011; Dungait et al., 2012; Moni et al., 2012). Through the application of two physical fractionation procedures we were able to obtain complementary information on aggregate size and stability, showing that the size of aggregates separated by aggregate size fractionation increased following forest expansion, while stable aggregates from size-density fractionation stored lower relative and absolute SOC amounts in forest than in grassland (PAPER II). Aggregate size fractionation cannot isolate "true aggregates", but rather a mixture of aggregates and POM (Moni et al., 2012). Stable aggregates separated through a relatively simple size-density fractionation approach better qualified as indicators of aggregate stability and showed high sensitivity both to variations in SOC allocation and stocks following forest succession on former grasslands (PAPER III). Even though stable aggregates can be considered a heterogeneous fraction in term of composition and stability, and therefore do not qualify as a unique model pool (Magid et al., 2010; Moni et al., 2012; Poeplau and Don, 2013), they represented a useful measurement tool for management-induced changes in SOM (PAPER II and III). The POM fraction separated by size-density fractionation is mainly composed of uncomplexed

OM derived from recently incorporated residues (Gregorich et al., 2006), and it is typically considered a labile SOC pool, younger than mineral associated OM and with turnover times from years to decades (von Lützow et al., 2007; Leifeld et al., 2009). However, POM showed longer turnover times in alpine soils than in warmer climates, likely due to environmental limitations (Leifeld and Fuhrer, 2009; Budge et al., 2011).

Chemical extraction can isolate OM components that are easily decomposable outside the soil, such as carbohydrates. However, these compounds showed a mean age similar or even greater than bulk SOM, as they can be preserved for many decades through stabilization with the mineral soil phases or their C can be subject to continuous microbial recycling (Derrien et al., 2006; Rumpel et al., 2010; Schmidt et al., 2011). Our study showed a positive relationship between carbohydrates, especially those with microbial origin, and aggregate stability (PAPER IV). The lower abundance of microbially-derived carbohydrates in forest sucessional stages suggested a possible explanation for the observed decrease in aggregate stability following forest expansion on grassland. Aggregate stability is enhanced by carbohydrates of microbial origin (Tisdall and Oades, 1982; Dungait et al., 2012), which can be stabilized within the soil matrix to a greater extent than plant-derived carbohydrates thanks to their capacity to bind to soil minerals (Kiem and Kögel-Knabner, 2003; Rumpel et al., 2010). The greater abundance of microbially-derived carbohydrates therefore was indicative not only of greater microbial OM processing but also of greater physical SOC protection afforded by stable aggregates in grassland compared with forest successional stages (PAPER IV).

Thermal analysis of bulk soil and physical soil fractions has shown promising results for monitoring SOM stability (Lopez-Capel et al., 2005; Leifeld et al., 2006; De la Rosa et al., 2008). Thermal stability was used as a proxy for SOM decomposability (Plante et al., 2011; Peltre et al., 2013; Leifeld and von Lützow, 2014). Peltre et al. (2013) and Plante et al. (2011) showed that high thermal stability corresponded to high biological stability (low respiration rates during incubation) in low-C soils (< 30 g C kg^{-1}), where organo-mineral association are the major SOM stabilization mechanism. On the contrary, in high-C soils (> 30 g C kg^{-1}) the SOM oxidized at relatively high temperatures (345-460°C) was related to low biological stability, likely due to the abundance of coarse plant debris, which are thermally resistant but at the same time easily biodegradable (Peltre et al., 2013). Interestingly, our study showed an inverse relationship between thermal stability and physical SOC protection, indicating that thermally labile SOM components corresponded to greater SOM stability and vice versa, consistent with findings of Peltre et al. (2013) for high-C soils (PAPER IV). Moreover, high abundance of thermally labile components corresponded to high carbohydrate content, suggesting a potential link between thermal and chemical analysis. The linear regression between peak height 1 (DSC) and soil carbohydrate content explained around 50% of the total variance, as other thermally labile compounds (e.g. aliphatic compounds) may oxidize at lower temperatures (PAPER IV). We also showed a significant and positive relationship between thermally labile to resistant SOM components ratio and the abundance of carbohydrates of microbial origin (i.e. rhamnose and galactose) (PAPER IV). Therefore, higher shares of thermally labile compounds can be indicative of high abundance of carbohydrates, especially of microbial origin, that in turn stabilize soil aggregates and may lead to long-term SOM stability. The positive relationship between labile SOM components (carbohydrates and thermally labile) and stable aggregates revealed by our study confirms the hypothesis by Cotrufo et al. (2013), according to which labile OM inputs are ultimately the major precursors of stable SOM.

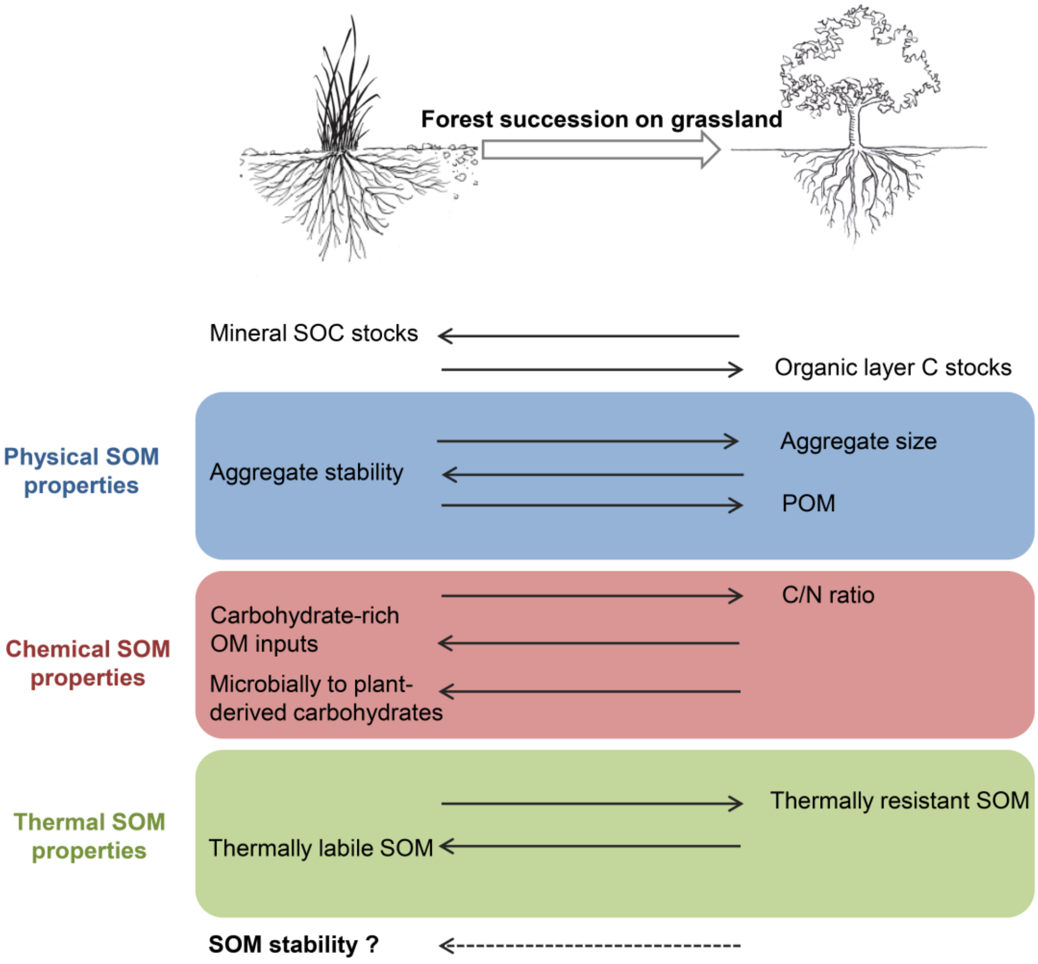

Fig. 10 A synthesis of changes in SOC stocks and physical, chemical and thermal SOM properties observed following forest succession on mountain grassland

Considering SOC stability as mainly determined by occlusion and adsorption mechanisms (i.e. physical SOC protection) rather than by intrinsic molecular recalcitrance (Dungait et al., 2012), we interpreted the decrease in physical SOC protection within the soil layers (lower mineral SOC stocks, higher organic layer SOC stocks) and within the mineral soil (lower stable aggregate-C, higher POM-C) following forest expansion on former grasslands as indicative of lower long-term SOC stability (Fig. 10). While plant-like SOM components were prevalent (high C/N, low microbially to plant-derived carbohydrates, high thermally resistant SOM) in forest successional stages, SOM components showed greater microbial processing (low C/N, high microbially to plant-derived carbohydrates, high thermally labile SOM) in grassland successional stages. The lower share of microbially-derived OM in forest successional stages together with the lower aggregate stability suggested that microbial OM processing is a major driver of physical SOC protection in the forest succession on grasslands. Our study therefore represents an important step toward a better understanding of SOM stabilization mechanisms. It provides a comprehensive view of changes in SOC stocks and in their properties, integrating all these aspects in a unifying framework for forest succession on grasslands.

Perspectives and future research

Forest expansion on grasslands

The impact of forest colonization of mountain grasslands on SOC was poorly understood, especially concerning physical SOC protection and mechanisms leading to these changes. This PhD thesis aimed to fill this gap of knowledge, considering that it is likely that large areas of agricultural lands in mountainous regions will be abandoned over the next decades and that forest will take over the abandoned areas. We showed that forest expansion on grasslands affected C distribution within the soil layers, which became richer in organic layer C but lower in mineral SOC stocks. The SOC stock changes shown by this study are in agreement with studies carried out in similar, mountainous environments. This confirmed that our study area, even though relatively small, was reasonably representative of forest expansion on mountain grasslands. Within the mineral soil, physical SOC protection declined, likely due to lower microbial OM processing, as indicated by lower shares of microbially-derived compounds. The decreased stable aggregate-C while increased POM-C revealed in this study, carried on fine-textured soils, is in line with studies comparing grassland and forest sites across a broad range of soil types. However, further studies should be carried out to specifically investigate the effects of this ongoing and widespread land-use change on SOC stocks and protection, in order to upscale results to the alpine region.

Fractionation procedures to investigate SOC stability

No single physical, chemical or biological fractionation procedure can adequately describe the continuum of SOM components, separating SOM into discrete fractions defined by specific stabilization mechanisms without causing SOM alteration (Olk and Gregorich, 2006; von Lützow et al., 2007; Plante et al., 2011). Our study integrated multiple approaches, using two physical soil fractionation procedures, chemical and thermal analyses. We were therefore able to investigate a range of SOM properties within a grassland-to-forest transition. However, this in-depth approach may not be feasible for the identification of changes in SOC stabilization in large-scale studies. Research needs to provide simple and reliable indicators of SOC stability, which could be used for SOC accounting purposes, in addition to measurements of bulk SOC contents.

Physical fractionation of SOM can elucidate the temporal variations of SOC to land-use and management changes, therefore several procedures have been developed. However, physical fractionation procedures often require high workloads and unknown degrees of uncertainty due to soil mass loss and misplacement among fractions. We optimized and evaluated a relatively simple size-density fractionation procedure. This procedure showed high repeatability and sensitivity to SOC changes following forest succession on grasslands, and has the potential to be applied to a wide range of land uses, soil types and environments. The fractions obtained through physical fractionation should adequately describe soil processes, therefore further testing is required to relate the C associated to physical fractions to easily decomposable C, estimated through laboratory incubations or field studies.

Thermal analysis can be used for the characterization of SOC stability, as it integrates chemical composition, molecular characteristics and interactions with the mineral phase. Moreover, as thermal analysis is fast and requires little sample preparation, thermal properties have the potential to be used as indicators of SOC stability. To successfully interpret thermal properties, however, we should verify the existence of reliable relationships with physical, chemical and biological characteristics of SOM across a wide range of soil types and environments (Plante et al., 2011; Peltre et al., 2013; Leifeld and von Lützow, 2014). However, it will be a challenge to exactly quantify the specific OM compounds that burn at certain temperatures and, even more interestingly, to build a straightforward and mechanistic relationship between thermal properties and SOC stability.

Global warming and SOC fractions

The effect of global warming on SOC is currently under debate and contradictory results were shown for temperature sensitivity of different SOC fractions (von Lützow and Kögel-Knabner, 2009; Conant et al., 2011; Schmidt et al., 2011). According to Arrhenius kinetics, temperature sensitivity of decomposition is higher in colder climates and for organic compounds with high activation energy (“stable”) than for compounds with low activation energies (Bosatta and Ågren, 1999; Leifeld and Fuhrer, 2005). Even though the contribution of stable SOC decomposition to total SOC decomposition is thought to be small, the increase in stable SOC decomposition may result in a considerable impact on atmospheric CO_2 concentrations, if we assume that OM inputs are not affected by climate change. However, several interacting mechanisms govern the responses of SOC to changes in soil temperature (Davidson and Janssens, 2006; Schmidt et al., 2011) and the effect of temperature on specific stabilization processes are difficult to identify (Conant et al., 2011). Long-term bare fallow experiments showed a positive relationship between temperature sensitivity and SOC stability (Lefèvre et al., 2014), confirming what has been found by the majority of incubation studies, i.e. stable SOC is more temperature sensitive than labile SOC (Conant et al., 2011). As in Lefèvre et al. (2014), long-term experiments (or naturally occurring chronosequences, as in our study) should be investigated to verify the responses of SOC stocks to climate change, trying to disentangle the several processes involved. Analysis of the molecular composition and physical protection of SOC fractions should be combined with measurements of their turnover time and their responses to changing climatic conditions (e.g. through laboratory and field incubations).

Throughout this PhD, I learned that the main characteristic of SOM resides in its complexity and in its paradoxical behaviors. Different interacting mechanisms contribute to its stabilization, and we are not yet fully able to disentangle or quantify them. These complex and fascinating interactions will certainly continue to drive research on SOC stocks and stability in the years to come.

Conclusions

Forest expansion contributed positively to atmospheric CO_2 removal, mainly through the C sequestered in tree biomass and organic layers, while the C stored in the mineral soil decreased from grassland to forest successional stages. Moreover, the physical SOC protection within the mineral soil declined, as shown by decreased SOC in stable aggregates while increased POM-C, suggesting that SOC can become more susceptible to management and environmental changes following forest succession on grasslands. The fractions separated through a relatively simple size-density fractionation were better suited than aggregate size fractions for the detection of SOC changes. Among size-density fractions, stable aggregates were a useful measurement tool for management-induced changes in SOC content and in its physical protection.

Carbohydrate and thermal analyses indicated higher shares of labile C within the bulk soil in grassland abandoned since 10 years than in managed grassland and forest-dominated successional stages. The ratios between microbially to plant-derived carbohydrates and thermally labile to resistant components decreased following forest expansion on grassland, and corresponded to a decrease in aggregate stability. This provided a possible explanation for the observed decrease in physical SOC protection following forest succession on grassland and suggest a major role of labile SOM components of microbial origin into soil aggregation and long-term SOM stability.

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