Dynamics of soil carbon, nitrogen, and phosphorus in calcareous soils after land-use abandonment – A chronosequence study

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Abstract

Aims The objective of this study was to investigate changes in soil total organic C (TOC), total nitrogen (TN), phosphorus (P) fractions, and microbial community structure during secondary succession after abandonment of vineyards on calcareous soils.

Methods Two chronosequences covering 200 years and differing in aspect and slope were established in Hungary, and the upper 10 cm of the mineral soils were studied.

Results We found strong increases in TOC concentrations after land-use abandonment, especially at the south-exposed sites. The TOC/TN ratio increased by a factor of 1.3 in the south-west exposed chronosequence and by a factor of 1.6 in south exposed chronosequence. The concentration of labile P ($NaHCO_3$-extractable P)

diminished during the first 50 years after land-use abandonment, leading to low P availability at the later stages of the succession. The total organic P (TOP) concentration increased during the first 40 years after abandonment. At the later stages of succession, TOP concentrations decreased again, while the ratio of TOC/TOP increased continuously over 200 years. The ratio of arbuscular-mycorrhizal-fungi-to-bacteria (AMF/bacteria) increased strongly during the first decade after abandonment of the vineyards.

Conclusions Our study indicates that impacts of former cultivation on secondary ecosystems persisted for more than a century, and that especially P concentrations showed long lasting legacy effects.

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Introduction

Former arable land-use can have strong legacy effects on secondary ecosystems (Dupouey et al. 2002; Foster et al. 2003; McLauchlan 2006). During the last decades many agricultural sites have been abandoned especially in Eastern Europe (Ramankutty 2006; Ramankutty and Foley 1998; Kurganova et al. 2010, 2014), and in little accessible areas such as terraced fields and vineyards (Dunjó et al. 2003; Koulouri and Giourga 2007; Zornozoa et al. 2009; Tarolli et al. 2014). Effects of land-use history on carbon (C), nutrients, vegetation,

and the microbial community may persist for more than a century (Dupouey et al. 2002; Foster et al. 2003; McLauchlan 2006; Harris 2009). Hence, for conservation management of secondary ecosystems it is important to know about signatures of past human activities and their longevity in order to be able to establish long-term management strategies (Poschlod and WallisDeVries 2002). The effects of land-use abandonment on soil C sequestration have been studied intensively during the last two decades (for reviews see Post and Kwon 2000; Guo and Gifford 2002). However, the effects of former cultivation on nutrient concentrations (McLauchlan 2006) and on the microbial community structure (van der Waal et al. 2006) have received considerably less attention. A thorough understanding of legacies of former types of land-use that included fertilization on soil nutrients is crucial for the management of secondary ecosystems since high levels of nitrogen (N) and phosphorus (P) can strongly reduce plant diversity and affect biogeochemical cycles (Gough and Marrs 1990; Stevens et al. 2004; Wassen et al. 2005).

Chronosequence approaches have been used frequently to investigate changes in vegetation and soil properties during secondary succession after land-use abandonment (Zak et al. 1990; Hooker and Compton 2003; van der Waal et al. 2006; Allison et al. 2007; Kalinina et al. 2009; Baer et al. 2002; Kalinina et al. 2011). The basic idea of this approach is to substitute space for time and to study a range of sites that constitute a timeline of successional states (Bardgett et al. 2005; Walker et al. 2010). Generally, it has been observed that after cessation of cultivation the vegetation reverted to a plant community similar to comparable non-disturbed sites. In temperate ecosystems with forest being the natural vegetation, the succession proceeded from agricultural site, to grassland, then to shrubland, and ultimately to forest (Knops and Tilman 2000; Hooker and Compton 2003; Poulton et al. 2003; Kalinina et al. 2009, 2011). Studies that considered long-term changes reported that it took almost 200 years in sandy soils of the taiga (Kalinina et al. 2009), and 400–500 years in prairie soils (Matamala et al. 2008) until the soils reached C stocks in the range of comparable non-disturbed sites. Total organic C (TOC) and total nitrogen (TN) accumulation after abandonment of land use were usually strongly related, and TOC/TN ratios increased due to increases in the C/N ratio of the inputs (Zak et al. 1990; Knops and Tilman 2000; Poulton et al. 2003; Matamala et al. 2008).

The few existing studies on the development of soil P concentrations during secondary succession showed that former arable land use led to increased total P and labile inorganic P concentrations due to fertilization, which persisted even several decades after land-use abandonment (Dupouey et al. 2002; Falkengren-Gerup et al. 2006). In a meta-analysis MacDonald et al. (2012) showed that labile and total P concentrations were elevated after land-use abandonment compared to non-cultivated soils, but reduced compared to soils that remained under agricultural use. In chronosequence studies, it has been shown that labile P concentrations decreased after cessation of cropping of sandy taiga soils and also after the establishment of forests on old fields in Europe (Kalinina et al. 2009; De Schrijver et al. 2012). In contrast, total organic P (TOP) concentrations increased during secondary succession of forests in Europe and in New Zealand due to increasing TOP inputs in the form of plant litter (Brandtberg et al. 2010; De Schrijver et al. 2012). Still more research is needed to gain insights into P dynamics after land-use abandonment, especially on calcareous soils, which strongly bind P due to precipitation of phosphate with calcium (Matar et al. 1992; Hinsinger et al. 2001).

Soil microbial community structure is strongly shaped by soil pH and soil TOC/TN ratios (Fierer et al. 2009). It has been reported that abandonment of agricultural production, leading to increases in soil TOC/TN ratios and decreases in soil pH, caused not only changes in microbial biomass C, but also in the microbial community structure (Allison et al. 2005; van der Waal et al. 2006; Zornoza et al. 2009). The abundance of saprotrophic fungi typically increases with the soil TOC/TN ratio, because C-rich organic material usually contains a lot of lignin, which can only be decomposed by fungi (van der Waal et al. 2006; Fierer et al. 2009). However, other authors reported small or no changes in microbial community structure following land-use abandonment (Potthof et al. 2006; Dickie et al. 2011). Some of the non-consistent results might be due to the use of a single phospholipid-derived fatty acids (PLFA) biomarker (18:2w6) for different taxonomic groups of fungi such as saprophytic fungi, arbuscular mycorrhizal fungi (AMF), and ectomycorrhizal fungi. This is problematic since the abundance of these different groups of fungi might undergo different dynamics. While ectomycorrhizal fungal biomass increase with the abundance of trees, AMF biomass decreases with abundance of trees

(Johnson et al. 1991). Thus, the dynamics of soil microbial community structure during secondary succession still requires further investigation with a more accurate separation of different groups of fungi.

The objective of this study was to investigate changes in soil TOC, TN, P fractions, and microbial community structure during secondary succession after abandonment of vineyards on calcareous soils in Tokaj, Northeast Hungary. For this purpose, we established two chronosequences that differed in aspect and slope. Tokaj was chosen as a study region because the vineyards in Tokaj are several hundred years old, and the area of abandoned vineyards has been continuously increasing, which is a pre-requisite for a chronosequence study. First, we hypothesized that after abandonment of the vineyards, soil TOC concentrations and TOC/TN ratios increase because we assumed increasing amounts of litter inputs and increases in woody vegetation with high C/N ratios after land-use abandonment. Our second hypothesis was that labile P concentrations decrease quickly due to plant P uptake, and that TOP concentrations increase because of increases in the inputs of organic P in form of plant litter. Third, we hypothesize that the fungi/bacteria ratio increases during secondary succession because of increases in the soil TOC/TN ratios.

Material and methods

Study area

The study area is located on Tokaj Nagy-Hill in Northeastern Hungary (48°06-07′ and 21°21-23′). The annual mean temperature is 8.5 °C, and the annual mean precipitation is about 600 mm. The Tokaj Nagy-Hill (21.08 km^2) rises from the alluvial plain (92–95 m above sea level) of the Hungarian Lowland up to 514 m above sea level. It is formed from late Miocene pyroxene dacite lava flows, and subordinately pyroxene dacite tuffs. The volcanic rocks are covered by Pleistocene aeolian loess sediments and loess derivates. The thickness of loess sediments and loess derivate layers varies between a few cm and 20 m with an average shallowness of 3–5 m. Vine production on the Nagy-Hill started in the late Iron Age, and became the dominant land-use type in the early Medieval Age. At the end of the 18th century, the entire hill was covered by vineyards. Since 2002 the Tokaj Wine Region is inscribed in the World Heritage List as a Historic Cultural Landscape because it represents a distinct viticulture tradition that has existed for at least a thousand years. Active vineyards have received P fertilizer of about 20 kg P_2O_5 ha^{-1} y^{-1} during the last 40 years. Before this, the vineyards were dominantly fertilized with manure and plant detritus. It can be assumed that the fertilizer application in the second half of the 20th century was very similar in all vineyards since they were owned and managed by the state. Due to various socioeconomic reasons many vineyards have been abandoned since the 19th century. The abandoned sites are undergoing a secondary succession and give habitat to many endemic plant and animal species (Sendtko 1999), which led to the designation of the area as a natural reserve since 1986.

Site selection

Land use information in historical maps and remotely sensed data was used to identify the extension of cultivated vineyards at different times in the past. The following maps were analyzed: I. Military Survey (1783; scale: ca. 1:28 800), II. Military Survey (1858; scale: 1:28 800), III. Military Survey (1884; 1: 25 000), Topographic maps from period of World War II (1940; 1:50 000), New Survey Topography Map (1960; 1: 25 000), Topography Map (1989; 1:10 000), satellite survey from (2010). Maps were partially available in digital form; others had to be digitized to compare using GIS software. All vectorized land use data were transformed into the same projection system (Unified National Projection, EOV) and the elevation deformation of air photographs was offset. Subsequently, vectorized land-use maps were created reflecting the land-use information for each time section using the software Quantum GIS 1.7 and Erdas Imagine 8.5. The extent of cultivated vineyards was identified, and compared with the extent depicted on the next oldest map. A decrease in the extensions of the area of cultivated vineyards was interpreted as abandonment of cultivation. Six ages of abandoned vineyards could be identified: 193±38, 142±13, 101±28, 63±10, 39±15, and 14±11 years. Additionally, we selected three active vineyards. The uncertainty (given as ±) is calculated as half the time between the releases of two consecutive maps. Based on elevation contour levels of the topographic map 1:10 000 we compiled a slope grade and a slope aspect map of the hill. By cross sectioning the slope grade-map, the

slope aspect map and the abandonment-age map, we marked out two time series (chronosequences) of sampling sites, situated on slopes with similar grade and aspect, but with decreasing age of abandonment. One chronosequence was marked out on 25–35 % slope facing south, and a second chronosequence was established on 17–25 % slope exposed to southwest. All sites were located in an area of 4 km². The maximum difference in height between the sites is 248 m in the south-facing chronosequence, and 312 m in the southwest exposed sites (Table 1).

Sampling and vegetation analysis

At each site, the uppermost 10 cm of the mineral soil were sampled in three plots of 10×10 m in late March 2013. In each of these three plots, one mixed sample composed of 10 single samples was collected using an auger. Additionally, we collected a mixed sample per site in three active vineyards also using an auger. The results of the analysis of these three samples from the vineyards currently in use were later used to calculate a mean representing the active vineyard soils. In order to determine the bulk density, three separate samples per site were taken using volumetric cylinders. The vegetation was analyzed in June 2013 in three 5× 5 m plots on each site. All aboveground visible plant

species were identified and the number of species and the covers of herbaceous, shrub, and canopy layers were determined at each site.

Soil chemical analysis

Samples were sieved field moist through a 2-mm sieve. After a pre-treatment with hot H_2O_2, texture was analyzed by sieving and sedimentation in Atterberg cylinders. For all further chemical analyses the samples were dried at 40 °C. The pH was determined in water (in a ratio 1:2.5) with a pH Ag/AgCl electrode. The carbonate concentration was measured gas volumetrically after treatment with HCl using a Scheibler Apparatus (Wertheim). TOC and TN were determined with an element analyzer (Vario EL, Elementar). Total P (TP) was extracted by pressure digestion in concentrated nitric acid according to Heinrichs et al. (1986), and was measured by an ICP-AES (Spectroflame, Spectro). Total Organic P (TOP) was determined according to Saunders and Williams (1955). Briefly, 1 g soil was extracted in 0.5 M H_2SO_4 on a shaker for 16 h. A second g soil was ignited at 450 °C over night and was also extracted in 0.5 M H_2SO_4 on a shaker for 16 h. Inorganic P was determined colorimetrically in both extracts with the malachite green method. The difference between the inorganic P in the extract of the ignited

Table 1 Aspect and slope, time since abandonment, elevation, soil density, pH, carbonate-C concentration, and the TOC, TN, and TP stocks in the uppermost 10 cm of the soils

Aspect and slope	Time since abandonment [years]	Elevation a.s.l. [m]	Soil density [g cm⁻³]	pH$_{H2O}$	Carbonate-C [g kg⁻¹]	TOC [kg m⁻²]	TN [g m⁻²]	TP [g m⁻²]
South-Southwest 25 %	0	114	1.10	8.3	8.4	0.99±0.28	121.1±20.0	74.3±3.0
South 25–35 %	14±10	114	1.22	8.5	16.8	1.18±0.14	113.2±3.0	77.8±6.5
	39±14	155	1.07	8.2	20.1	3.76±0.12	308.7±17.8	64.7±1.6
	63±10	233	0.94	8.3	11.4	3.29±0.31	264.0±21.6	32.0±2.3
	101±28	203	1.01	8.1	2.4	4.39±0.34	354.0±16.1	44.5±2.2
	142±13	305	0.81	6.1	8.8	3.27±0.20	269.1±16.3	37.1±3.8
	193±38	362	0.75	8.0	6.8	3.75±0.65	282.5±41.3	21.5±2.6
Southwest 17–25 %	14±11	257	1.25	8.4	6.9	0.91±0.25	121.8±24.9	71.2±14.5
	39±15	236	1.00	8.2	6.6	2.22±0.43	206.3±26.8	53.6±1.3
	63±10	275	1.15	8.2	9.5	1.88±0.07	197.1±11.4	55.1±4.1
	142±13	377	1.14	8.4	1.9	1.73±0.35	183.9±24.6	55.3±3.1
	193±38	426	1.07	6.8	8.4	3.45±0.21	304.6±14.2	59.4±3.1

Values represent means calculated from three independent samples per site, and±depict standard deviations

and the non-ignited soil was considered as the total organic P (TOP). Olsen P was extracted in 0.5 M NaHCO₃ (in the following P_{NaHCO_3}) for 16 h on a shaker according to Olsen et al. 1954), and was determined colorimetrically with the malachite green method (van Veldhoven and Mannaerts 1987). The NaHCO₃-extractable P represents a labile P fraction in alkaline soils.

Soil microbial analyses

Microbial C was determined according to Vance et al. (1987). Five g sieved, field moist soil were fumigated with chloroform for 24 h and was subsequently extracted in 0.5 M K₂SO₄ for 1 h on a shaker. Another 5 g were directly extracted in 0.5 M K₂SO₄ for 1 h on a shaker. The organic C in both extracts was determined with a CN Analyzer (Multi 2100, Analytik Jena) after acidification to pH 3 in order to remove inorganic C. The difference between the organic C in the extract of the fumigated and the non-fumigated soil was multiplied by the conversion factor 2.64 for C (Vance et al. 1987), and the result of this multiplication is considered as the microbial C.

Soil microbial community structure was analyzed by phospholipid-derived fatty acids (PLFA). Phospholipids were extracted and purified from field moist soil according to Frostegård et al. (1991) modified as described in Gunina et al. (2014). For measurements on a GC, fatty acids were saponified to free fatty acids and derivatized into fatty acid methyl esters (FAME), which were measured using a GC–MS (Triple Quad, Agilent Technologies 7000, Agilent) with a HP-5MS column (Agilent). Recovery efficiency was determined with two internal standards, of which one was added at the beginning of the extraction and the other prior to the derivatization in order to correct for losses during the extraction. PLFAs were assigned to taxonomic microbial groups in the same way as in Allison et al. (2005) (see Appendix 1). The total PLFA concentration was calculated as the sum of the concentrations of the PLFAs that were identified (Appendix 2). We calculated the fungi/bacteria ratio from the abundance of the fungal PLFA biomarker (18:2ω6,9), representing all fungi, and the abundance of all bacterial biomarkers. The arbuscular mycorrhizal fungi (AMF)/bacteria ratio was calculated from the abundance of the AMF biomarker (16:1ω5c) and the abundance of all bacterial biomarkers.

Data analysis and statistics

All element ratios were calculated on a mass basis. Means and standard deviations were calculated from three independent samples per site. TOC concentration and TOC/TN ratio as a function of time since abandonment were approximated by an asymptotic concave function according to McMahon et al. (2010):

$$Y = Y_{max} \frac{A}{K + A}$$

A is the time since land-use abandonment, Y_{max} is the maximum TOC concentration or C/N ratio, respectively, and K is a constant that corresponds to the age required to reach half Y_{max}.

The P_{NAHCO3} concentration as a function of time since abandonment was approximated with a monoexponential model:

$$Y = ae^{-bx}$$

a represents the P_{NaHCO3} concentration, k is a rate constant, and x is the time after land-use abandonment.

Spearman's correlation coefficients were calculated, and the significance of the correlations was tested by the Spearman's test. All data analysis was done using R environment for statistical computing (R Core Team 2013).

Results

In both chronosequences, the vegetation in the abandoned vineyards developed from grassland to shrubby grassland, to shrubland, to forest (Appendix 2). While drought-tolerant, xerothermic species (*Quercus cerris*, *Quercus pubescens*, *Pinus nigra*, *Fraxinus ornus*, *Rosa gallica*, *Amygdalus nana*, *Euonymus verrucosus*; Appendix 2) dominated at the sites of the south-facing chronosequence, moist-favoring species dominated at the southwest facing chronosequence sites (*Fraxinus excelsior*, *Robinia pseudo-acacia*, *Juglans regia*, *Populus canescens*, *Eleagnus angustifolia*; Appendix 2). The texture of the soils at all sites was silty loam. All soils contained large concentrations of carbonate leading to alkaline soil pH, except for two soils that had lower carbonate concentrations and lower soil pH (Table 1).

TOC concentrations increased in the soils of both chronosequences with time after abandonment (Fig. 1a). The TOC concentration increased more strongly at the south-exposed than at the southwest-exposed sites, leading to a 1.6-times higher TOC concentration at the longest-abandoned south-facing site compared to the longest-abandoned, southwest-exposed site. Considering the TOC stocks (Table 1), we observed an increase by a factor of 3.5 and 2.1, respectively, in the uppermost 10 cm of the soils.

The TOC concentration was linearly positive correlated with the TN concentration ($r=0.99$, $p<0.05$), indicating a close relation between TOC and TN accumulation. TOC/TN ratios of each chronosequence could be approximated with an asymptotic concave function (Fig. 1b), showing a quick increase in the TOC/TN ratio during the first 40 years after abandonment of the
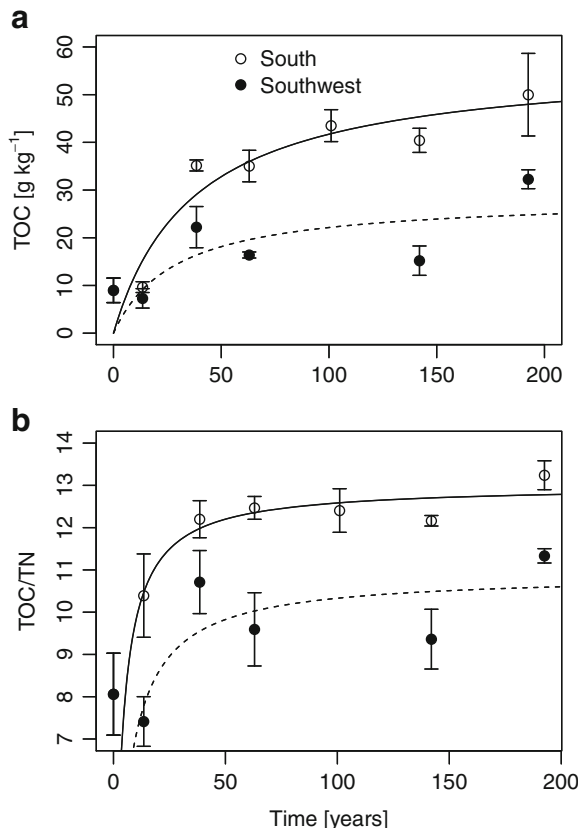
vineyards by a factor of 1.3 and 1.6, respectively and no further changes during the following decades.

The P_{NaHCO3} concentrations varied largely between the active vineyard soils (Fig. 2a). The P_{NaHCO3} concentration decreased during the first 50 years after abandonment of the sites, and stabilized at a low level thereafter (Fig. 2a). While the P_{NaHCO3} concentration decreased, the TOP concentration increased during the first 40 years
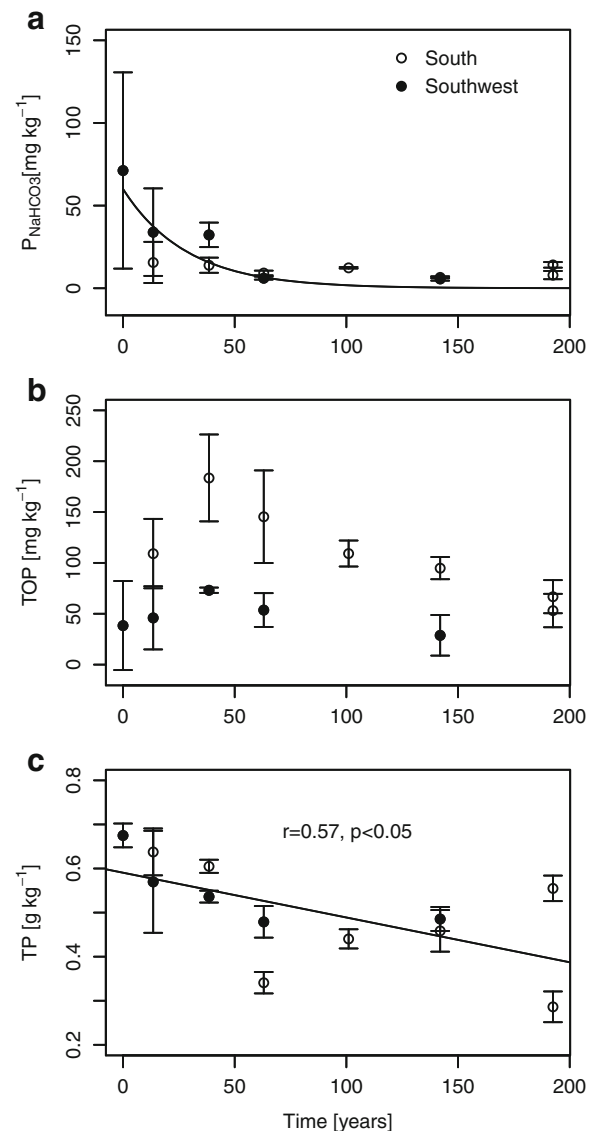

Fig. 1 TOC concentrations (**a**) and TOC/TN ratio (**b**) in the south- and southwest-facing soils as a function of time since abandonment of the sites. The observational data was fitted with an asymptotic concave function. Points represent means calculated from three independent samples per site. *Error bars* depict standard deviations

Fig. 2 Concentrations of Olsen P (P_{NaHCO3}; **a**), total organic P (TOP; **b**), and total P (TP; **c**) in the south- and the southwest-facing soils as a function of time since abandonment of the sites. Points represent means calculated from three independent samples per site. *Error bars* depict standard deviations. r is the Spearman's correlation coefficient

after abandonment, and decreased at the later stages of the secondary succession (Fig. 2b). The TP concentration was slightly negatively correlated with the time since abandonment of the sites ($r = 0.57$, $p < 0.05$; Fig. 2c). The TOC/TOP ratio increased linearly after cessation of agriculture ($r = 0.85$, $p < 0.05$; Fig. 3). It had increased by a factor of 7 after 193 years, and seemed still to be rising at the time of analysis (Fig. 3).

The microbial C concentration was significantly positive correlated with the TOC ($r = 0.83$, $p < 0.05$), the TN ($r = 0.81$, $p < 0.05$), and with the total PLFA concentration ($r = 0.90$, $p < 0.05$). Neither bacteria nor AMF changed significantly with time since abandonment ($p > 0.05$). However, the active vineyards had a significantly lower AMF/bacteria ratio than the recently abandoned sites (Fig. 4). If the active vineyards were not considered, the AMF/bacteria ratio decreased from 0.20 to 0.07 during secondary succession ($r = 0.92$, $p < 0.05$, Fig. 4). The ratio of fungi/bacteria did not change significantly during secondary succession ($r = -0.50$, $p > 0.05$).

Discussion

Here we studied the long-term succession of vineyards after their abandonment based on two chronosequences.

Fig. 4 The AMF/bacteria ratio in the soils of both chronosequences as a function of time since the abandonment of the sites. Points represent means calculated from three independent samples per site. The data from the active vineyards was not included for the calculation of the linear correlation. r is the Spearman's correlation coefficient

The chronosequence approach is associated with some uncertainty since many details about the historical land-use are unknown and agricultural practices have changed over time. However, since there is hardly any other way to gain knowledge about long-term development of soils, this approach offers valuable insights.

TOC

We found a strong increase in TOC stocks during secondary succession (Table 1). This is in accordance with previous studies, which showed that TOC stocks in soils generally increased after land-use abandonment (Knops and Tilman 2000; Hooker and Compton 2003; Poulton et al. 2003; Kalinina et al. 2009, 2011). The asymptotic shape of the increase in the TOC concentration (Fig. 1a) suggests that TOC accumulation slowed after an initial succession phase associated with high TOC accumulation rates that lasted approximately 60 years in the case of the south-exposed sites, and 80 years in the case of the southwest-exposed sites. At the south-facing sites, we found a stronger increase in TOC concentrations than at the southwest-exposed sites (Fig. 1a), which can likely be attributed to the drier microclimate, but might also be due to differences in organic matter inputs. The increase in TOC stocks by 210 and 350 % during the secondary succession is relatively large. Based on a meta-analysis, Poeplau et al. (2011) reported increases
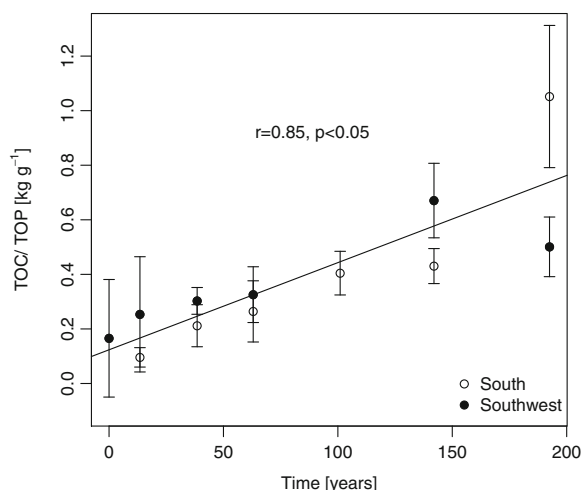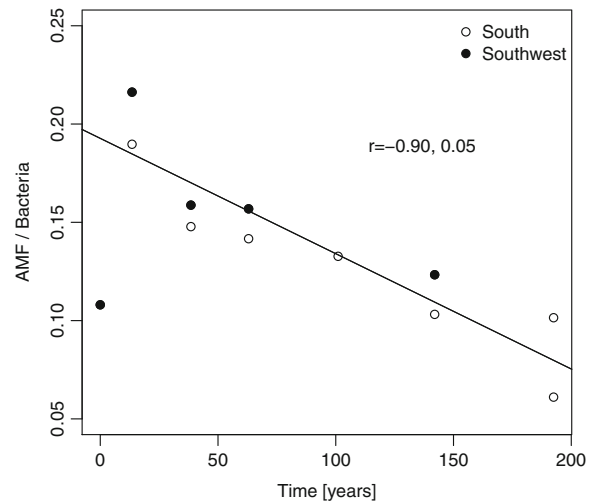
Fig. 3 The TOC/TOP ratio in the soils of both chronosequences as a function of time since abandonment of the sites. Points represent means calculated from three independent samples per site. *Error bars* depict standard deviations. r is the Spearman's correlation coefficient

in TOC stocks by 116 % after land-use conversion from cropland to forest, and by 123 % after land-use conversion from cropland to grassland in the top 30 cm of soils of the temperate zone. The reason why the increases observed here seem to be much larger is that we only report results for the top 10 cm. If we considered also the subsoil, the total changes would be smaller since TOC concentrations in the subsoil tend to change less after land-use conversion than in the topsoil (Guo and Gifford 2002). Our findings suggest that the topsoils of former vineyards can sequester large amounts of organic C after abandonment. This is an important finding because vineyards in many parts of Europe have been abandoned during the last century (Dunjó et al. 2003; Ramankutty and Foley 1998). In this context, our results indicate that these post-agricultural soils turned into C sinks.

Soil stoichiometry I: TOC and TN

The linear relation between the TOC and the TN concentrations of both chronosequences suggests that TOC and TN accumulation were related in the same way in both chronosequences independently of microclimatic conditions. Approximately 40 years after cessation of cultivation, the TOC/TN ratios did not increase any more (Fig. 1b), suggesting that the system had reached a new equilibrium. The reason for the generally low TOC/TN ratios is most likely that herbaceous plants were still very abundant even at the later stages of the succession (Appendix 2).

Poulton et al. (2003) showed based on archived samples that TOC accumulation rates strongly increased during secondary succession of N depleted soils after a certain soil TN concentration had been reached. From this finding the authors concluded that the secondary succession was initially limited by N, and shifted from N to another limitation – potentially light – once N had accumulated due to N_2-fixation and atmospheric N deposition. In contrast to Poulton et al. (2003), we observed that initially TOC accumulated faster than TN (Fig. 1b), indicating that at the early stage of succession, plant biomass production was not N limited, likely because plants could still draw on fertilizer-derived N. Similarly, a close relation between TOC and TN accrual, and an initial increase in the TOC/TN ratio was observed by Zak et al. (1990) in soils of abandoned fields.

Soil stoichiometry II: TOC and P

The slight decrease in the TP concentrations (Fig. 2c) is in accordance with the results of a meta-analysis, which showed that former agricultural soils contained higher TP concentrations than non-agricultural reference soils, and that TP concentrations slowly decreased with time after abandonment (Mac Donald et al. 2012). The fast decrease in the P_{NaHCO3} concentration (Fig. 2a) is in accordance with previous studies that observed a relatively quick depletion of labile P after land-use abandonment. De Schrijver et al. (2012) reported a decrease in the P_{NaHCO3} concentration by 50 % during 50 years of post-agricultural forest development in central Europe. In sandy taiga soils, a decrease in labile P concentrations in the topsoil by even 90 % during the first 50 years after land-use abandonment was observed (Kalinina et al. 2009). The reason for the decrease in the P_{NaHCO3} concentration is most likely that labile phosphate, which might ultimately be derived from fertilizer, precipitated with calcium. The binding of phosphate to calcium is a typical process occurring in all calcareous soils (Matar et al. 1992; Hinsinger et al. 2001). Since the soils under study contain high amounts of calcium carbonate (Table 1), it seems likely that this process strongly contributed to the decrease in labile P. A second reason for the decrease in labile inorganic P might be the uptake of labile P by plants. However, while these two explanations for the decrease in labile P concentration in time seem likely, we cannot exclude that the P_{NaHCO3} concentrations were higher in the younger sites of the chronosequence because the sites had received larger amounts of fertilizer. The low P_{NaHCO3} concentration after 50 years of land–use abandonment indicates a very low P availability. P_{NaHCO3} concentrations in the same range have been observed in the top cm of calcareous grasslands soils in Germany (Alt et al. 2011). Many calcareous soils on hillslopes and in little accessible areas in Europe have been abandoned during the last decades (Dunjó et al. 2003; Koulouri and Giourga 2007; Ramankutty and Foley 1998; Poschlod and WallisDeVries 2002; Tarolli et al. 2014), giving space to secondary plant succession. Our findings suggest that in calcareous soils, labile P concentrations decreased quickly after cessation of cultivation, leading to low P availability, and thus, allowing growth of species that are sensitive to high soil P availabilities (Wassen et al. 2005).

The initial increase in the soil TOP concentration (Fig. 2b) supports the interpretation that labile P was

taken up by the vegetation during the first decades following the cessation of agriculture, leading first to an increase in the plant P concentration and later to an increase in the soil TOP concentration due to litter inputs. The observation that the TOP concentrations were higher in the south than in the south-west facing soils is in accordance with the higher TOC concentrations in these soils. An increase in the TOP concentration has also be observed by De Schrijver et al. (2012) during 50 years of post-agricultural forest development in central Europe and by Brantberg et al. (2010) during forest development in New Zealand on bare soil.

After the initial increase in TOP concentrations, TOP decreased again after about 40 years in the secondary succession studied here. An initial increase in TOP concentrations and a decrease at the later stages of development has also been reported in the upper 10 cm of soils undergoing primary succession in New Zealand and Hawaii (Richardson et al. 2004; Crews et al. 1995). Moreover, an increase in the soil TOC/TOP ratio over the course of succession, similar to the one observed here (Fig. 3), has also been found during primary succession in a chronosequence in New Zealand (Richardson et al. 2004). To our knowledge, our study is the first to show that this dynamic occurs during secondary succession in a similar way as it occurs during primary succession. The chronosequence of soils undergoing secondary succession studied by De Schrijver et al. (2012) only covered 50 years of soil development. Thus, it might be that the TOP concentration in these soils also decreases at later stages of succession and that the chronosequence studied by De Schrijver et al. (2012) just did not cover the timespan necessary to observe it. Hence, these results underline the importance of studies of the long-term development of ecosystems after land-use abandonment. The decreases in TOP concentrations at the later stages of the succession might have two reasons; increased soil organic P mineralization and increasing C/P ratios of the litter inputs at the later stages of succession (Richardson et al. 2004; Crews et al. 1995). The interpretation that the decrease in TOP is not only controlled by litter inputs but also by mineralization of organic P is supported by the often observed increase in soil phosphatase activity with decreasing P availability (Olander and Vitousek 2000; Spohn and Kuzyakov 2013a). While an initial increase in TOP concentration and a subsequent decrease in TOP at later stages of succession is well documented for primary succession, this is the first

study showing this dynamic to occur also during secondary succession after land-use abandonment.

Microbial community composition

A close relation between microbial C and TOC, and a slightly smaller correlation between microbial C and TN as observed here has also been found in previous chronosequence studies on secondary succession (Zak et al. 1990; Zeller et al. 2001; Allisson et al. 2005), indicating that the saprophytic microbial community is primarily C limited. Generally, saprophytic soil microorganisms in the temperate zone are thought to be C limited (Scheu and Joergensen 1999; Demoling et al. 2007). Even in temperate soils, in which plants might be P limited, the soil microbial community was C limited, and organic P mineralization seemed to be rather driven by microbial need for C rather than for P (Spohn and Kuyzakov 2013b; Heuck et al. 2015).

Harris (2009) developed a conceptual model, stating that during ecosystem succession from an industrially degraded or agricultural site to pioneer vegetation, to grassland, to shrubland, to finally forest, the fungi/bacteria ratio increases continuously caused by the increasing C/N ratio of the organic matter inputs. The microbial biomass, in contrast, increases first, reaches a maximum in the grassland because of extremely high rhizodeposition and decreases during the following succession towards forest (Harris 2009). Our findings do not support this conceptual model since, first, we did not find a decrease in microbial biomass from the grasslands to the forest, and second, we did not observe differences in the fungi/bacteria ratio. In contrast, we found that the microbial community composition did not change with respect to the total fungal biomass during secondary succession, while it did shift with respect to the relative abundance of AMF (Fig. 4). The results indicate that AMF reacted more sensitively to the increase in woody vegetation than other groups of fungi such as ectomycorrhizal and saprophytic fungi. The strong decrease in AMF in relation to bacteria (Fig. 4) can be attributed to an increase in abundance of trees that do not form symbiosis with arbuscular mycorrhizal fungi but with ectomycorrhizal fungi. However, the correlation between the AMF/bacteria ratio and the vegetation cover of all layers was not significant ($p < 0.05$), suggesting that either these parameters do not represent well the belowground abundance or that also additional factors impacted the AMF/bacteria ratio. The large increase

in the AMF/bacteria ratio from the active vineyard soils to the recently abandoned soils (Fig. 4) suggests that AMF recovered very quickly after cessation of cultivation. This is most likely due to the fast increase in the abundance of herbaceous plants. However, more research is needed to confirm this finding.

The lack of change in the total fungi/bacteria ratio might be due to the relatively small change in the TOC/TN ratio, and the overall relatively low TOC/TN ratios even at the long-abandoned sites. The relative abundance of fungi usually increase with the soil TOC/TN ratio (Fiere et al. 2009). Here we observed, only relatively small changes in the TOC/TN ratios, which seems to be one reason for why we did not see a change in the total fungi/bacteria ratio. Next to the soil TOC/TN ratio, soil microbial communities are strongly affected by soil pH, and alkaline pH usually favors bacteria over fungi (Fierer et al. 2009). The soils of the two chronosequences were almost all alkaline, and no consistent change in soil pH has been observed, which is most probably a second reason for the stable fungi/bacteria ratio. A third reason for why the relative abundance of the total fungal biomass did not change might be that vineyards hardly ever get ploughed. Ploughing has a strong impact on fungal hyphae and it is assumed that a low fungal biomass in most croplands can largely be attributed to the physical disturbance caused by regular ploughing (Young and Ritz 2000). Viniculture likely does not affect the total fungi/bacteria ratio as strongly as other forms of arable land-use since it does not include ploughing. Consequently, no recovery of the fungi/bacteria ratio can be observed during secondary succession of former vineyard soils.

Conclusion

As hypothesized, we observed increases in TOC concentrations and TOC/TN ratios during the secondary succession of both south- and southwest-exposed sites, indicating that the post-agricultural soils turned into C sinks. We found that labile P concentrations decreased during the first 50 years after land use abandonment most probably due to sorption and plant uptake, while TOP concentrations initially increased as expected. The TOP concentrations decreased again 50 years after cessation of viniculture. Together with the increasing TOC/TOP concentrations this indicates high organic P mineralization rates at the later stages of the succession and

presumably litter inputs with lower C/P ratio. Taken together, our results indicate that P dynamics during long-term secondary succession are similar to the dynamics during primary succession, but occur over a shorter period of time. The third hypothesis did not hold true; against our expectations, we observed no change in the fungi/bacteria ratio, but a decrease in the AMF/bacteria ratio that was most probably caused by the increasing abundance of trees. In conclusion, our study shows that impacts of former cultivation on secondary ecosystems may persist for more than a century, and that especially P concentrations show long lasting legacy effects.

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