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REGULAR ARTICLE

Influence of deep soil horizons on Ca nutrition of forest stands along a loessic soil sequence

Thomas Drouet · Jacques Herbauts · Daniel Demaiffe

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Abstract
Background and aims Acid leached soils developed on loessic materials in Central Belgium present homogenous edaphic characteristics and similar patterns of strontium isotopic composition (^{87}Sr/^{86}Sr), used as tracer of the origin of calcium. This was inconsistent with the large range of ^{87}Sr/^{86}Sr ratios measured in leaves from beech stands developed on the sites. We hypothesised that the deep carbonate-bearing horizon (>2.5 m) with low ^{87}Sr/^{86}Sr ratio, could be a complementary source of Ca for tree nutrition.
Methods We studied the change in foliar Sr isotopic composition and element concentrations in 12 forest sites along a soil sequence. This soil sequence was selected to include the largest range of variations in the depth at which the calcareous loess horizon occurs. In complement, root depth development was determined in six sites down to 300 cm.
Results Our results reveal that Sr originating from deep carbonate influences significantly the isotopic composition of beech trees growing on loessic soils. This influence contributes from 20 to 80 % to the Ca nutrition of trees depending on their position along the soil sequence.
Conclusions Despite its deep location in the soil profile, the carbonate-bearing horizon is determinant for the nutrient status of these forests.

Keywords Calcium · Carbonate · *Fagus sylvatica* · Forest soils · Loess · Soil sequence · Strontium isotopes · Tree nutrition

Introduction

The two main sources of Ca in tree nutrition are mineral weathering release and atmospheric inputs. Natural Sr isotopes are commonly used to distinguish between these Ca nutrient sources in forest ecosystems (Åberg et al. 1990; Dambrine et al. 1997; Blum et al. 2002, 2008; Poszwa et al. 2004; Drouet et al. 2005a; Bern et al. 2005). Strontium, which is ubiquitous in nature and one of the most abundant trace elements in surficial deposits and rocks, acts as a proxy for Ca because both are alkaline earth element with similar ionic radius and the same valence (Capo et al. 1998). Barium, another alkaline earth element, has also been used as a tracer of Ca cycling (Bullen and Bailey 2005), but divergent behaviour with Ca reduces this application (Drouet and Herbauts 2008).

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T. Drouet (✉) · J. Herbauts
Laboratoire d'Ecologie végétale et Biogéochimie (CP 244),
Université Libre de Bruxelles (ULB), B-1050 Bruxelles,
Belgium
e-mail: tdrouetd@ulb.ac.be

D. Demaiffe
Laboratoire de Géochimie (CP 160/02), Université Libre de
Bruxelles (ULB), B-1050 Bruxelles, Belgium

In a previous study, we used a mixing equation based on the ^{87}Sr/^{86}Sr ratio of bulk precipitation (atmospheric end-member), of 0.1 M HCl-extractable soil fraction (weathering end-member) and of European beech (*Fagus sylvatica* L.) wood and foliage (mixing compartment) to determine the relative Ca contributions of the sources in two forest stands of Central and High Belgium (Drouet et al. 2005a). We concluded that the Ca atmospheric contribution was high and of the same order of magnitude in the two forest sites (on average, 75 and 78 %, in Central and High Belgium, respectively), in spite of very different total and exchangeable Ca contents in their soils (the Albeluvisols on loess of Central Belgium has 30 times more exchangeable Ca than the Cambisols on Devonian shales of High Belgium). We concluded that the Ca atmospheric contribution was not influenced by the soil Ca reserves. We subsequently extended these analyses to four other beech stands on analogous soil profiles (Albeluvisols, WRB 2006) and similar parent material as in the former site studied (Drouet et al. 2007b). Results showed a remarkably similar vertical distribution of the Sr isotopic ratios within the different soil profiles down to 2 m depth, both for the bulk soil and for the exchangeable fraction. By contrast, isotopic measurements made on beech leaves growing on these soils revealed a broad range of variation of the ^{87}Sr/^{86}Sr ratio (from 0.711240 to 0.712134). This variation in the mixing compartment was surprising because the beech stands were developed in very similar environmental, edaphic and nutritional conditions. Great similarities between the selected forest stands were suggested by: (1) a largely similar ground vegetation, dominated by acidity indicatory species associated with humus of the moder-mor type, attesting a widespread oligotrophic character of these forest sites; (2) a unique type of soil (Albeluvisol) for all the forest stands with nearly constant physico-chemical properties down to 2-m depth and a strong desaturation of the exchange complex to around one metre depth (Drouet et al. 2007b); (3) element composition of beech leaves (the unique or dominant tree species) not significantly different between sites.

Several studies have shown that forests on nutrient poor temperate soils rely on atmospheric inputs of Ca and efficient recycling to maintain productivity (Blum et al. 2008, 2012; Farkaš et al. 2011; Van der Heijden et al. 2013) whereas other studies described the influence of weathering sources often situated at depth (~1 m) (Dijkstra and Smits 2002; Poszwa et al. 2004; Berger et al. 2006). In few studies, the use of multitracer techniques were used to disentangle the role of more than two sources of Ca in forest ecosystems (Blum et al. 2002; Porder et al. 2005; Drouet and Herbauts 2008; Bélanger et al. 2012).

The aim of the present study is to determine which environmental parameters could explain the contrasting ^{87}Sr/^{86}Sr ratios measured in beech trees growing on a homogeneous parent material (Pleistocene loess deposit) with very similar physico-chemical and mineralogical properties in the upper two metres of the profile. We hypothesised that the mineral nutrition of trees depends on more than one weathering source and that the Sr isotopic composition of the trees could be influenced by the access to distinct soil horizons with contrasting isotopic signatures. The hypothesis of several weathering end-members in these loessic soils is supported by the fact that a carbonate-bearing layer is often preserved at variable depth in the lower part of the soil profiles. The $CaCO_3$ fraction of the unweathered loess is characterized by low Sr isotopic ratio (^{87}Sr/^{86}Sr= 0.708255) which strongly differs from that of the alumino-silicated matrix of the loess (^{87}Sr/^{86}Sr>0.713; Drouet et al. 2005a, 2007b). The variable depth at which this carbonate-bearing layer occurs at the different sites could therefore modulate the tree root access to a soil horizon with a very contrasting Sr isotopic ratio compared to that of the overlying horizons.

To test this hypothesis, 12 forest sites were selected to cover a sequence of soils developed from thick to thin loess deposits, and, at the extreme, to sandy and glauconite-rich outcrops of Tertiary marine sediments. On thick loess deposits, soils were selected to range from profiles with a shallow (~180 cm) calcareous C_k horizon to profiles where this horizon is much deeper (~350 cm); in some sites this carbonate-bearing layer is totally absent. A pot experiment was conducted to complete the sequence and mimic the situation (which does not occur in the studied area) in which beech trees are growing on a calcareous loess substrate. In each stand, the main physico-chemical and mineralogical characteristics of the soil and of the parent material were determined, together with the measure of the ^{87}Sr/^{86}Sr ratio and/or the Ca and Sr contents in vegetation (beech leaves, litter and roots) and in major soil horizons (acid extractable, exchangeable and water extractable fractions).

Materials and methods

Sites description

The 12 forest stands selected for this study are all located in the Soignes Regional Forest, southeast of Brussels. This forest covers 4400 ha on a loessic plateau (at about 120 m above sea-level) that belongs to the loess belt of Central Belgium. The climate is of humid temperate type, with average annual rainfall and temperature of 780 mm and 9.8 °C, respectively. The natural vegetation is a deciduous forest with European beech and pedunculate oak as co-dominant species. Beech has been however extensively planted since the end of the 18th century and most sites of our study are even-aged beech high forests, mainly planted between 1803 and 1865 (Fig. S1). The ground vegetation is largely indicative of acid soil conditions and is dominated by moder-mor and acid mull indicatory species (Herbauts et al. 1996).

The soil parent material is a Pleistocene niveo-aeolian loess deposit, several meters thick, overlying Tertiary marine sediments, mainly Upper (Priabonian) and Middle (Lutetian) Eocene sands and clayey sands, often glauconite-bearing. The transition zone between the loess deposit and the Tertiary substratum frequently contains allochtonous broken flints, indicating a pre-Quaternary erosion surface. The upper metres of the loess are dated from the end of the Weichselian glaciation. The unweathered loess contains around 10 % $CaCO_3$ and is frequently preserved in the lower part of the loess deposit, generally at a depth greater than 250 cm (Fig. S1). The thickness of the unweathered loess is highly variable, from few decimetres to several metres. In some sites, the loess mantle is completely decarbonated down to its contact with the Tertiary substratum, at several metres in depth (Van Ranst et al. 1982; Langohr and Sanders 1985; Herbauts et al. 1996; Brahy et al. 2000; Drouet et al. 2007b).

Loess deposits underlie about 90 % of the forests in the surrounding area, and form acid leached soils with an $A_hEB_{t(g)}C$ profile (Fragic Cutanic Albeluvisols, WRB 2006). These acid soils are low in base saturation to about one metre depth. On the sandy Tertiary deposits which locally outcrop due to Quaternary erosion (~1 % of the forested area), mainly on topographic highs (generally above 100 m altitude), soils are either podzolic with an $A_hEB_hB_sC$ profile (Albic Rustic Podzol) or hydromorphic acid leached on more clayey sands with an $A_hE_gB_{tg}C$ profile (Fragic Cutanic Stagnic Albeluvisol). Both are also strongly acid and deeply desaturated.

Sampling

Twelve forest sites were selected to cover a sequence of soils developed on Pleistocene loess substrate with variable thickness from ~250 cm to more than 600 cm, from deep to shallow loessial deposits and, at the extreme, to sandy outcrops of glauconitic Tertiary sediments (Fig. S1). The 12 sites are *Saint Hubert II*, *Tambour*, *Mésanges*, *Relais des Dames II*, *Comte de Flandre I*, *Tir aux Pigeons*, *Saint Hubert I*, *Tumuli*, *Moulin*, *Comte de Flandre II*, *Diependelle* and *Léonard* sites, hereafter HUB II, TAM, MES, REL II, CTE I, PIG, HUB I, TUM, MLN, CTE II, DIEP and LND, respectively. Nine sites are beechwood stands developed on deep loessic soil profiles. In eight of these sites, the unweathered calcareous loess (C_k horizon) is preserved at the lower part of the soil profiles, but at very contrasting depths from 180 to 340 cm. In one site (MLN), the loess deposit is free of any calcareous layer in spite of its great thickness (~350 cm before the contact with the Tertiary substratum). The soil sampling was completed with three forest sites (two beech stands and a mixed beech and Scots pine plantation) where the progressive shallowing of the loessial deposit (from 200 cm in the CTE II site to less than 50 cm in the DIEP site) leads to the outcropping of Tertiary sands in LND site.

Within each forest stand, soil samples were taken from soil pits or by augering. Major soil horizons were systematically sampled (ten replicates for A_h, five for other horizons): A_h (0–5 cm), E (5–25 cm), B_{21t} (55–75 cm), B_{22t} (100–120 cm) and B_{31t} (175–200 cm) horizons in the Fragic Albeluvisols; A_h (0–5 cm), E_g (5–25 cm), B_{21tg} (55–75 cm) and B_{31tg} (175–200 cm) horizon in the Stagnic Albeluvisol; A_h (0–10 cm), E (10–20 cm), B_h (30–40 cm), B_s (50–70 cm) and C (80–100 cm) horizons in the Albic Rustic Podzol. When present, the carbonate-bearing C_k horizon (unweathered loess) was also sampled at the base of the niveo-aeolian deposit (>180 cm).

Within most of the forest stands, the mean depth at which the calcareous loess (C_k horizon) or the underlying Tertiary deposit occurs in the lower part of the soil profiles was determined by five core samples, in the same area as those used for leaf sampling. The thickness of the calcareous layer was also evaluated by coring.

Within each forest site, sun leaves were collected in the crowns of five beech trees (height of 6 to 8 m) during the month of September of three different years, following the site. In October 2006, ten samples of freshly-fallen leaf litter were sampled on the ground of each site including a deep carbonate-bearing layer to assess the chemistry of the entire leaf production along the depth gradient of the calcareous layer. The depth reached by beech roots was determined in six sites (HUB II, TAM, MES, REL II, CTE I, PIG) by five soil coring down to 320 cm. In the MES site, roots <4 mm from soil cores (770 cm^3) were sampled to quantify the root distribution and for chemical analyses.

Sample preparation and analytical methods

Soil samples were air-dried and sieved to a particle size of <2 mm. Particle-size distribution was determined by the pipette method after H_2O_2 pretreatment and dispersion with Na-citrate. Common methods were used to determine exchangeable acidity and exchangeable aluminium (1 M KCl extraction; derivative titration curve for H^+ and Al^{3+}), exchangeable cations (1 M CH_3COONH_4 pH 7 extraction; ICP-OES determination of Ca, Mg, K, Ba and Sr concentrations), carbonate (dry combustion; Ströhlein dosimeter). Effective base saturation (%) was determined as the sum of cations (Ca, Mg, K) divided by the effective cation-exchange capacity (sum of cations and exchangeable acidity).

The soil exchangeable fraction, defined as the sum of cations present in the soil solution and of cations adsorbed on mineral and organic soil colloids, was extracted with suprapure CH_3COONH_4 (10 g soil with 50 mL 1 M CH_3COONH_4 at pH 7). The extracts were dried on a hot plate, and ashed in covered zirconium crucibles (16 h at 450 °C) to eliminate acetate. Ashes were dissolved with 1 mL suprapure HCl on a hot plate.

The acid extractable fraction which simulates natural release by weathering, was obtained by selectively dissolving the more weatherable minerals (Blum et al. 2002; Drouet et al. 2007b). Prior to this acid treatment, exchangeable cations were leached with three successive suprapure 1 M CH_3COONH_4 solutions and the residue was rinsed three times with distilled water. Four successive acid extracts were obtained from the residue by shaking 5 g of soil with 50 mL suprapure 0.1 M HCl during 2 h, centrifugating and filtering to 0.45 μm. Surface horizons containing organic matter (i.e. A_h and E horizons) were at first treated with hot H_2O_2 (Merck, suprapur grade) and afterwards leached with suprapure 1 M CH_3COONH_4 to eliminate exchangeable and organically-bound cations. Carbonate of the C_k layer was selectively dissolved in a single step by suprapure 0.5 M CH_3COOH (Drouet et al. 2005b). Glauconite grains were separated from the sandy material of the DIEP site, sonicated in distilled water and totally dissolved following a procedure described in Drouet et al. (2007b). Water extracts of soil with a 1:1 volume ratio were obtained by shaking of 20 g soil sample with 20 mL distilled H_2O (18 MΩ cm) during 1 h, centrifugation and filtration to 0.45 μm. Calcium, Mg, K, Ba and Sr concentrations of the extracts were determined by inductively coupled plasma emission spectroscopy (Vista-MPX CCD simultaneous ICP-OES, Varian, Australia).

Root samples (<4 mm) were rinsed with copious amounts of water, washed with successive agitation in demineralised water and methanol. To avoid contamination with fine soil particles (which could modify the root isotopic signature) root bark was stripped with a blade. It should be noted that this treatment causes a decrease of the measured Ca/Sr ratio because bark has a high Ca/Sr ratio (Drouet and Herbauts 2008). All vegetation material was dried at 65 °C and ground in a Retsch ZM100 mill using a 750-μm screen. Mineralization of 1–2 g of plant sample was done by dry ashing in covered zirconium crucibles (16 h at 450 °C). Ashes were dissolved in suprapure HCl on a hot plate.

Pot experiment

A greenhouse pot experiment was performed to achieve the virtual condition in which a beech stand is growing directly on calcareous loess material. This simulation provides a supplementary point to the natural soil sequence. Beech seedlings were cultivated on a substrate made of one part of deep calcareous loess (10 % $CaCO_3$) mixed with five parts of acid-washed sand to facilitate water drainage. Five replicates were watered each week with a standard nutritive solution (Ingestad 1971) without Ca and Sr content. Major plant parts (leaves, shoots, roots, rootlets) were harvested for analyses after 120 days of cultivation. Replicates were analysed separately for nutrient concentration and leaf samples were bulked for Sr isotopic determination.

Sr isotope analyses

Chemical separation of Sr was carried out by cation exchange chromatography. Strontium isotopic compositions were measured on a VG Sector 54 multicollector thermal ionisation mass spectrometer. The measured ^{87}Sr/^{86}Sr ratios were normalized to ^{86}Sr/^{88}Sr=0.1194. Measurements of the NBS-987 Sr standard yielded on average ^{87}Sr/^{86}Sr value of 0.710281±0.000009 (2σ, *n*=35); isotopic values of the samples were not adjusted. Additional details on the analytical procedure can be found in Ashwal et al. (2002).

Calculations and statistical analyses

The proportion of Sr in a mixture derived from two sources is calculated using a two-component (1 and 2) mixing equation (Capo et al. 1998; Drouet et al. 2005a).

$$X(\mathrm{Sr})_1 = \frac{\left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_{\mathrm{Mix}} - \left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_2}{\left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_1 - \left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_2} \tag{1}$$

where $X(\mathrm{Sr})_1$ represents the mass fraction of Sr derived from source #1. The 1 and 2 subscripts refer to the two sources. The *Mix* subscript indicates the mixture component. The relative contribution of Ca from two sources to a mixing component is given by a mixing equation (Eq. 2):

$$X(\mathrm{Ca})_1 = \frac{\left[\left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_{\mathrm{Mix}} - \left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_2\right]/\left(\mathrm{Ca}/\mathrm{Sr}\right)_2}{\left[\left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_{\mathrm{Mix}} - \left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_2\right]/\left(\mathrm{Ca}/\mathrm{Sr}\right)_2 + \left[\left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_1 - \left({}^{87}\mathrm{Sr}/{}^{86}\mathrm{Sr}\right)_{\mathrm{Mix}}\right]/\left(\mathrm{Ca}/\mathrm{Sr}\right)_1} \tag{2}$$

where $X(\mathrm{Ca})_1$ represents the mass fraction of Ca derived from the atmospheric source. In order to solve complex systems involving more than two sources of Ca for plants, Ca/Sr ratio can be used as a second independent tracer. Both tracers can be plotted in a ^{87}Sr/^{86}Sr vs. Ca/Sr diagram to discriminate among several sources of Ca (Blum et al. 2002; Porder et al. 2005; Bern et al. 2005; Drouet and Herbauts 2008; Bélanger et al. 2012). These two ratios, by contrast with concentration values are not affected by element accumulation in the biomass and therefore reflect the mixing of the sources.

A correction of the Ca/Sr ratio was used for the leaf samples to take into account the physiological discrimination between Ca and Sr inside trees, from roots to leaves, through the transpiration stream. This discrimination process can be corrected by a discrimination factor (DF) which is constant for beech along a large range of soil chemical status (DF=2.99±0.60, mean±SD; Drouet and Herbauts 2008). This correction allows to estimate the Ca/Sr value at the level of root absorption and to plot root data on the same graph.

We used linear regressions to test for the relationship between the Sr isotopic ratio or element concentrations of tree leaves and the depth of the carbonate-bearing horizon. Results of the pot experiment were not included in the regression analyses. One-way ANOVAs were conducted on element concentrations (Ca, Sr, Mg, K and Ba) and element ratios (Ca/Sr, Ca/Ba) of leaves and leaf litter after logarithmic transformations to achieve the homogeneity of variance (Levene test) when necessary. Multiple mean comparisons between sites were subsequently made by the Tukey's HSD test. Comparisons of mean element concentrations of beech foliage in the sites examined with other beech stands on contrasting substrates (calcicolous and acidophilous) were applied with permutational *t*-tests after transformations to meet the requirement for homoscedasticity between the groups. Statistical analyses were performed with the R statistical software (R Development Core Team 2014).

Results

Control of the soil physico-chemical homogeneity along the soil sequence

Figure S1 displays the variation of the parent materials with depth along the soil sequence. The first eight profiles show a gradual deepening of the calcareous horizon from 180 to 350 cm depth. The last four profiles of the sequence show the progressive thinning of the loess deposit and the increasing thickness of the Tertiary material near the surface, the latter outcropping in the LND site. The distribution of some analytical properties common to the soil developed on loess material (first ten profiles of the soil sequence) are presented in Fig. 1.

Clay distribution pattern (Fig. 1a) is very uniform along the sequence. This confirms the homogeneity of the textural distribution through the profiles in link with the shared parent material and the same intensity of clay illuviation among sites. CTE II site is an exception, showing an increase of clay content down to 200 cm, which indicates the appearance of the Tertiary sandy-clay material at this depth. Clay percentage in the E horizon (9.9±1.7 %) increases to 19.0±2.2 % in the illuvial B_{21t} horizon and decreases gradually to a mean value of 12.6±1.1 % in the C_{k2} horizon.

The effective base saturation (Fig. 1b) also shows a remarkably reproducible profile of distribution with depth. A decrease is observed from the A_h to the E horizon (from 30 to 12 %) followed by a slight increase to 23 % in the B_{21t} horizon. A sharp increase of base saturation (from 23 to 70 %) begins between 55–75 and 100–120 cm depth. This indicates a radical change in chemical conditions between the B_{21t} and the B_{22t} horizons. Base saturation increases to a mean of 89 % at 175–200 cm (C horizon) and tends to 100 % below. This was previously interpreted as the remains of Ca released by the carbonate dissolution (Drouet et al. 2007a). The distribution of the effective base saturation among the different sites appears regular regardless of the depth of the carbonate-bearing horizon (180 to 350 cm depth). The pattern of exchangeable Ca through the profiles (Fig. 1c) is roughly similar to that of the effective base saturation. The sharp rise of Ca concentrations occurring below 200 cm corresponds either to the proximity with the carbonate-bearing C_k horizon (at 180 or 250 cm for the HUB II and MES sites, respectively) or to an accumulation of Ca derived from former loess-carbonate and adsorbed on the first cm of the clay-rich Tertiary material (CTE II site).

The distribution of the exchangeable Ca/Sr ratio is more variable from site to site (Fig. 1d). The A_h horizons of the TAM, HUB and MES sites (carbonate source <260 cm) present Ca/Sr ratios (275 to 321) which are higher than the mean (230±90) whereas CTE I, HUB I, TUM sites (carbonate source >290 cm) show values (103 to 172) clearly lower than the mean. The variability among sites declines with depth. The mean Ca/Sr value decreases to a minimum of 157±21 in the B_{22t} horizon and increases to 182±17 in the C horizon. A marked increase is observed in the vicinity of the carbonate-bearing layer (carbonate Ca/Sr=455) which occurs at contrasting depth, following the sites (Fig. S1). The increase of the Ca/Sr ratio is more gradual down to 350 cm for sites witch have deeper carbonate-bearing layer or which do not have such horizon (HUB I, MLN sites).

The pattern of the exchangeable Ca/Ba ratios with depth (Fig. 1e) is quite different from the Ca/Sr pattern: high mean Ca/Ba values are observed in the A_h horizon (Ca/Ba=90), there is then a sharp decrease in the E horizon (Ca/Ba=13) and a gradual increase from 7 to 39 from the B_{21t} down to the C horizon. Profiles are very similar from site to site down to this depth. Below

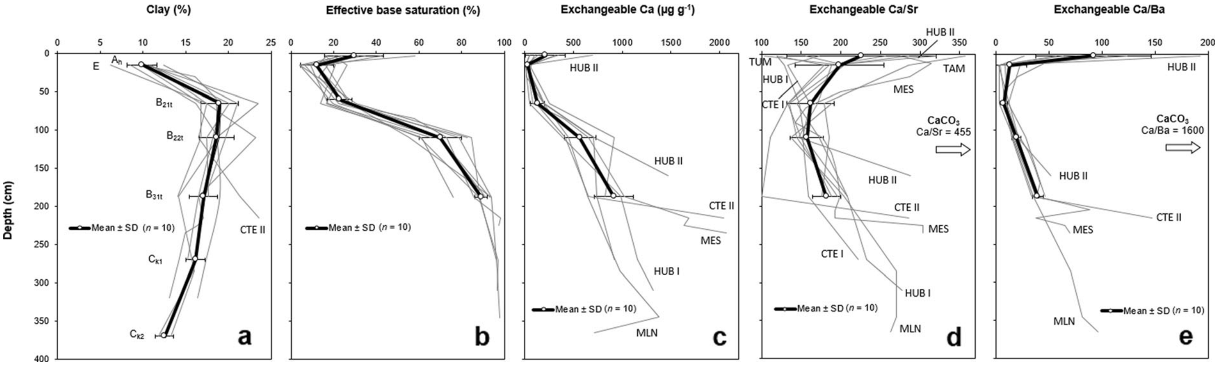


Fig. 1 Distribution of major soil parameters with depth in the 10 loessic soils (Alisol/Albeluvisols) of the sequence. Mean values± SD are presented for the soil horizons developed on non-calcareous loess material (n=8 to 10 depending on the horizon). **a** Clay content (%). **b** Effective base saturation (%). **c** Exchangeable Ca (µg g^{-1}). **d** Exchangeable Ca/Sr ratio. **e** Exchangeable Ca/Ba ratio. $CaCO_3$ represents the carbonate fraction of the C_k horizon extracted by selective dissolution

200 cm, divergence begins to be marked among sites with a steep increase for CTE II and a more gradual increase for MLN site. Exchangeable Ca/Ba values are always very far from that of the carbonate selective dissolution (Ca/Ba=1600) but tends to this value with depth.

Sr isotopic composition of the soil acid-extractable and exchangeable fractions

Soil Sr isotopic data of the loessic soils have partially been published and discussed by Drouet et al. (2005b, 2007b). Complementary measurements were made in five forest sites belonging to the soils studied previously and including soils developed on deep loessial deposits (CTE I, HUB II and MLN sites) and soils influenced by underlying Tertiary sediments (DIEP and LND sites).

In the Albeluvisols developed on loess, Sr isotopic ratios of the 0.1 M HCl extracts (simulating natural Sr release by selective dissolution of the most weatherable minerals) range from 0.712067 (B_{21t} horizon) to 0.713643 (A_h horizon) (Drouet et al. 2007b). In the present study we focused our attention on the B_{21t}, B_{22t}, B_{31t} and C horizons (at 55–75, 100–120, 175–200 and >200 cm depth, respectively), because these horizons were thought to take a prominent part in tree mineral nutrition and are also good indicators of the mineralogical composition of the parent material in the soil sequence. This last point is clearly demonstrated by the strong increase of the $^{87}Sr/^{86}Sr$ ratio of the 0.1 M HCl extracts from pure loess (mean values for the B_{21t} and the B_{31t}: 0.7125±0.0005 and 0.7137±0.0002, n=3, respectively) (Drouet et al. 2005a) to Tertiary glauconious sediments (0.721445 in the C horizon of the LND site). The relatively high $^{87}Sr/^{86}Sr$ ratio (0.716390) measured in the B_{21t} of a soil profile developed in a deep loess deposit (~350 cm deep), where no C_k horizon is present (MLN site), could be due to contrasting weathering conditions in a hydromorphous environment compared to those of the same horizon in other loessic soil profiles.

Drouet et al. (2005a) have shown that the distribution with depth of the Sr isotopic ratio of the exchangeable fraction in the Albeluvisols of the Soignes Regional Forest was very similar from site to site. Isotopic determinations of the B_{21t} horizon of two complementary forest stands, HUB II and CTE I sites (0.712776 and 0.712294, respectively), confirm that there is only a slight variation of the Sr isotopic composition of the exchangeable soil fraction between sites. Only in one soil profile (MLN site), the $^{87}Sr/^{86}Sr$ ratio of the B_{21t} horizon (0.721618) is clearly higher than in all the other loessic soils (mean $^{87}Sr/^{86}Sr$ ratio=0.7130±0.0002, n=5); the same observation was already noted for the HCl extracts. The slight variation of the isotopic composition between the different soil profiles clearly suggests that the soil forming processes, which govern the composition of the exchangeable pool in the soils developed on deep loess deposits, are very similar from site to site. When the loessic mantle is thin and strongly contaminated by the underlying Tertiary glauconious sediments, the $^{87}Sr/^{86}Sr$ ratio of the exchangeable pool is supplied by radiogenic Sr probably resulting from the weathering of micaceous minerals and it consequently increases sharply (0.718160 in the B_{21tg} horizon of the DIEP profile). However, glauconite has a rather low $^{87}Sr/^{86}Sr$ ratio (0.713141) and its content does not explain the high isotopic value of these horizons.

Element composition of beech foliage and leaf litter

Mean Ca, Mg, K and Sr concentrations and Ca/Sr ratios of beech foliage and leaf litter within the forest stands examined are given in Table 1. In the beech stands developed on loessic soils, Ca contents of green leaves are on average around 7500 μg g^{-1}, with values ranging from 6300 to 9500 μg g^{-1}. Foliage Sr concentrations vary between 9.0 and 16.3 μg g^{-1}. Young forest stands are characterized by high K and Mg foliar contents but have no particular pattern in Ca and Sr foliar content (PIG and TAM sites).

Differences among sites on deep loess are not significant (Tukey's HSD post-hoc test) for Ca and Sr concentrations in green leaves and litter except for the remote sites in the sequence (Table 1). By contrast, differences among sites are significant for leaf and litter Ca/Sr ratios. Calcium concentrations commonly found in beech leaves of Belgian forests on calcareous substrates range from 13,900 to 17,000 μg g^{-1} (Table 1) and are significantly higher (t-test, P >0.01) than beech leaves from sites with a deeper (>180 cm) carbonate-bearing layer. Also remarkable is that the Ca foliar contents of beech stands presenting a deep C_k horizon are significantly higher than those of typical acidophilous beech stands of High Belgium (t-test, P<0.01) which range from 3600 to 6000 μg g^{-1} (Denaeyer-De Smet and Duvigneaud 1977; Misson et al. 2001; Drouet and Herbauts 2008). Conversely,

Table 1 Concentrations of Ca, Sr, Mg, and K, Ca/Sr ratio and ^{87}Sr/^{86}Sr isotopic ratio in beech leaves and leaf litter of the soil sequence studied, in leaves of other Belgian forests, and in the pot experiment

Site	*n*	Ca µg g^{-1}±SD	Sr	Mg	K	Ca/Sr g g^{-1}±SD	^{87}Sr/^{86}Sr ±2σ×10^{-6}
Soignes soil sequence							
Leaves							
MES	5	9500±1300^{c}	13.7±2.0^{cd}	1500±200^{cde}	6300±600^{d}	699±50^{cd}	0.7112±0.0002^{c}
PIG	5	9200±2700^{cd}	16.3±5.4^{c}	2300±1100^{c}	11,000±2600^{c}	570±50de	0.711663±5
HUB II	5	8000±300^{cd}	13.3±1.7^{cd}	1700±200^{cd}	6000±1200^{d}	600±70^{cde}	0.710727±5
TUM	5	7900±700^{cd}	13.9±2.5^{cd}	1300±200de	7100±500^{d}	580±80de	0.712591±10
CTE I	5	7200±1800^{cd}	14.2±4.6^{cd}	1300±300de	5300±1300^{d}	520±100^{e}	0.712134±5
DIEP	5	7100±1500	9.5±2.1	1100±500	6500±1600	750±80	0.712744±6
TAM	5	6900±1200^{cd}	9.5±2.2^{d}	2000±600^{cd}	11,500±1100^{c}	740±70^{c}	0.711348±5
HUB I	5	6800±900^{cd}	11.1±2.5^{cd}	1300±400de	5600±1000^{d}	630±110^{cde}	0.711977±5
MLN	5	6400±1700	9.8±3.9	1000±200	5300±7200	690±140	0.711988±7
CTE II	5	6300±2000	12.8±3.1	1200±300	4600±500	480±60	0.712959±6
REL II	5	6300±1300^{d}	9.7±1.8^{d}	1000±200^{e}	6200±1100^{d}	640±40^{cde}	0.711688±7
LND	5	5400±1300	9.0±1.4	1000±300	8400±1200	600±130	0.713233±6
Leaf litter							
TAM	10	11,600±1000^{c}	17.1±1.9^{cd}	1200±100^{c}	1190±130^{cd}	680±30^{c}	
MES	10	10,300±1000^{cd}	16.8±1.6^{cd}	1130±120^{cd}	1230±170^{c}	610±20^{d}	
HUB II	10	9900±1200de	17.5±2.0^{cd}	950±120^{e}	990±150de	570±20^{ed}	
REL II	10	9900±1200de	16.9±2.1^{cd}	1000±170^{cd}	1030±160de	590±30de	
TUM	10	9900±700de	18.9±1.8^{c}	970±80de	970±100^{e}	520±20^{g}	
HUB I	10	9400±800de	17.1±0.8^{cd}	910±120de	1160±190^{cde}	550±30^{fg}	
CTE I	10	8900±700^{e}	16.5±1.1^{d}	880±100^{e}	1050±120^{cde}	540±20^{fg}	
Calcicolous beech stands							
Trois Fontaines(a)	5	15,200±7200	83.6±37.9	1700±500	6700±3200	180±6	
Bois de la Côte I(a)	5	17,000±4700	15.0±4.1	1800±400	7400±1200	1130±30	
Matagne(b)	5	13,900±2500	–	1400±300	8000±2000		
Virelles(c)		16,000	–	1300	11,000		
Mesophilous beech stands							
Bois de la Côte II(a)	5	10,300±1500	10.5±1.5	1800±400	3900±1200	980±60	
Acidophilous beech stands							
Poursumont(a)	16	6000±1700	11.3±3.5	1100±300	5200±1700	550±170	
Smuid(a)	10	5500±2000	14.6±4.9	1200±300	5200±1000	374±40	

Table 1 (continued)

Site	*n*	Ca µg g^{-1}±SD	Sr	Mg	K	Ca/Sr g g^{-1}±SD	^{87}Sr/^{86}Sr ±2σ×10^{-6}
Libin(b)	5	4900±900	–	1400±200	8000±750		
Mirwart(d)		5600	–	1600	8500		
Belgian Ardenne(e)		3600–5600	–	600–1300	5200–9100		
Greenhouse pot experiment							
Plantlet leaves	5	11,900±5900	19.6±7.5	1000±500	3400±1000	600±130	0.709025±9[b]
Shoot	5	13,700±5100	37.3±13.0	1700±800	4200±1500	370±40	
Roots	5	11,800±5100	33.1±13.2	2300±800	4900±1600	350±20	
Whole plantlet	5	12,000±1700	30.2±5.0	1700±300	4100±300	400±20	

Data are presented as mean±SD; *n* is the number of samples analyzed. Non-overlapping letters within colums designate statistically significant differences ($P<0.05$) between means (Tukey's HSD), only stands on loess are compared

[c] Mean of four individual tree measurements±SD; Drouet et al. 2005b

[b] Composite sample, $n=5$

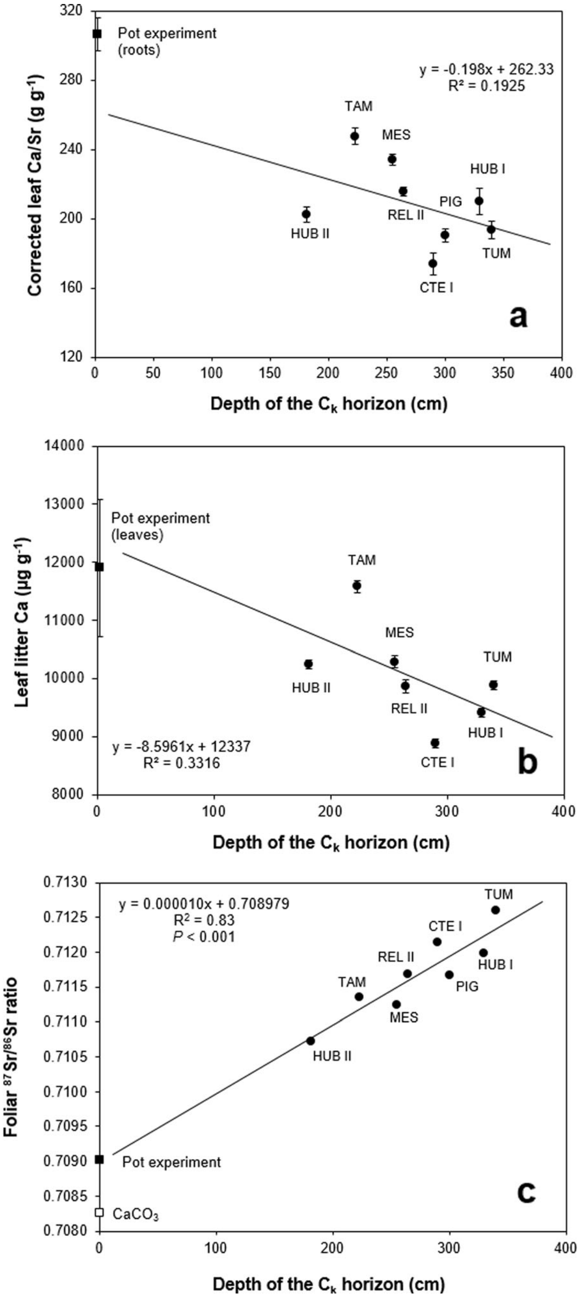
[c] Drouet and Herbauts 2008

[d] Herbauts, unpublished data

[e] Denaeyer-De Smet 1969

[f] Denaeyer-De Smet and Duvigneaud 1977

[g] Misson et al. 2001



◀**Fig. 2** **a** Relationship between the corrected foliar Ca/Sr ratio (see text for explanation) and the depth of the carbonate-bearing horizon along the soil sequence. MLN, CTE II, DIEP and LND sites are not represented because of the influence of the Tertiary substrate. **b** Relationship between the leaf litter Ca content and the depth of the C_k horizon. **c** Relationship between the foliar ^{87}Sr/^{86}Sr ratio measured on beech trees along a loessic soil sequence and the depth of the carbonate-bearing (C_k) horizon. Plotted on the y-axis of the 3 diagrams are respectively the Ca/Sr ratio of beech roots (**a**), the leaf litter Ca content (**b**) and the foliar ^{87}Sr/^{86}Sr ratio (**c**) of beech plantlets from pot experiment which simulate the case of a carbonate-bearing layer outcropping at the surface (*filled square*). These last points are not included in the regression analyses. The isotopic signature of the deep carbonate is also indicated in Fig. **c** (*open square*)

Ca contents of leaves from stands of the sequence without C_k horizon are not significantly different to that of acidophilous stands (High Belgium). The comparison of sites with and without C_k horizon revealed significant differences for Ca and Mg (*t*-test, $P<0.01$) as well as for Sr ($P<0.05$) and Ba concentrations in leaves ($P<0.05$). We observe a correlation between the corrected leaf Ca/Sr ratio and the depth of occurrence of the C_k horizon ($r^2=0.19$, $P<0.05$, $n=8$; Fig. 2a). It is interesting to note that root Ca/Sr value from the greenhouse experiment plots close to the intercept of the regression line with the *y*-axis (Fig. 2a). By contrast, no correlations were observed between the depth of the C_k horizon and the Ca, Sr, Ba concentration or the Ba/Ca ratio in green leaves. But regression analyses of litter data confirm the relationship between the depth of the C_k horizon (explanatory variable) and the litter Ca/Sr ratio ($r^2=0.29$, $P<0.001$, $n=7$) and show a negative relationship with the Ca/Ba ratio ($r^2=0.47$, $P<0.001$). These samples also revealed significant ($P<0.01$) negative relationships between leaf litter Ca ($r^2=0.33$; Fig. 2b), Mg ($r^2=0.09$), and Mn concentrations ($r^2=0.09$) with the depth of the carbonate-bearing horizon. A significant positive relationship was found between litter Ba concentration and the depth of the C_k horizon ($r^2=0.17$, $P<0.001$). No relationship was found between litter K and Sr concentrations and the explanatory variable.

Sr isotopic composition, Ca/Sr and Ca/Ba ratios of beech leaves and roots

The ^{87}Sr/^{86}Sr ratios of the beech leaves are given in Table 1; measurements show a wide range of variation, from 0.710727 to 0.713233. The lowest values (<0.7117) are typical of leaves collected in beech stands growing on loess deposits with a shallow calcareous C_k horizon (<260 cm: HUB II, TAM and MES sites). The highest values (>0.7127) characterize beech trees developed either on shallow loess deposits, underlain by Tertiary glauconious clayey sands (CTE II site) or directly on outcropping Tertiary marine sediments (DIEP and LND sites) (Fig. S1).

Intermediate values correspond to forest stands growing on deep loess deposits without C_k horizon (MLN site) or having a calcareous layer occurring at depths>260 cm (REL II, CTE I, PIG, HUB I and TUM sites). Leaves of beech seedlings cultivated directly on calcareous loess display a low Sr isotopic signature (0.709025) which is nevertheless slightly higher than that of carbonate (0.708255; Drouet et al. 2005a). Similarly to the Ca content in litter, there is a highly significant correlation ($r^2=0.83$, $P<0.001$, $n=8$) between the Sr isotopic composition of beech foliage and the depth at which the C_k horizon appears (Fig. 2c). Strikingly, the intercept of the regression line on the *y*-axis (which represents the foliar $^{87}Sr/^{86}Sr$ ratio in the case of a virtual calcareous layer directly outcropping at surface) indicates a Sr isotopic ratio (0.708979) very close to that measured on both beech leaves from pot experiment growing directly on calcareous loess (0.709025) and that of the $CaCO_3$ fraction of the unweathered loess (MES C_k horizon: 0.708255).

Figure 3 shows the variation with depth of the $^{87}Sr/^{86}Sr$ ratio and of the Ca/Sr and Ca/Ba ratios in soil exchangeable fraction, in soil water extracts and tree roots as well as the evolution of the root density through the soil profile. Except for the shallow A_h horizon, the Sr isotopic ratios of the exchangeable fraction are higher than that of the foliage in the whole profile down to a depth of 175 cm (Fig. 3a). Even if the data are more limited in depth, the same observation can be drawn from $^{87}Sr/^{86}Sr$ ratio of the exchangeable fraction in other sites (PIG, TUM, HUB II, not shown) and that of the soil solution in the MES site (Fig. 3a) published by Drouet et al. (2007b). The low foliar Sr isotopic ratio indicates that a significant part of the Ca taken up by trees occurs in deep layers with $^{87}Sr/^{86}Sr<0.711$, i.e. down to 175 cm depth. For both the exchangeable fraction and the soil solution, the $^{87}Sr/^{86}Sr$ ratios of the topsoil horizon are lower than those of the E and B_{21t} horizons and are most probably linked to the litter return which presents low ratios. The Sr isotopic signature of the roots broadly follows the general pattern of the exchangeable fractions but not closely.

The Ca/Sr ratios of the exchangeable fractions, the water extracts and soil solution are very comparable (Fig. 3b), which allows to exclude marked differences of sorption between Ca and Sr on the exchange complex. By contrast, the Ca/Sr ratios measured in roots do not follow the soil extractible fractions. This could be explained by the treatment of the root samples before analysis. The evolution of the Ca/Ba ratios (Fig. 3c) reveals a great difference between the soil exchangeable fraction and the water extracts, which confirms the high sorption of Ba on the exchange complex and its doubtful use in tracing studies (Drouet and Herbauts 2008). Figure 3d presents the profile of root density in the MES site. Greater root densities are observed in the 25–150 cm interval. For the MES site and five others, roots were detected down to 300 cm.

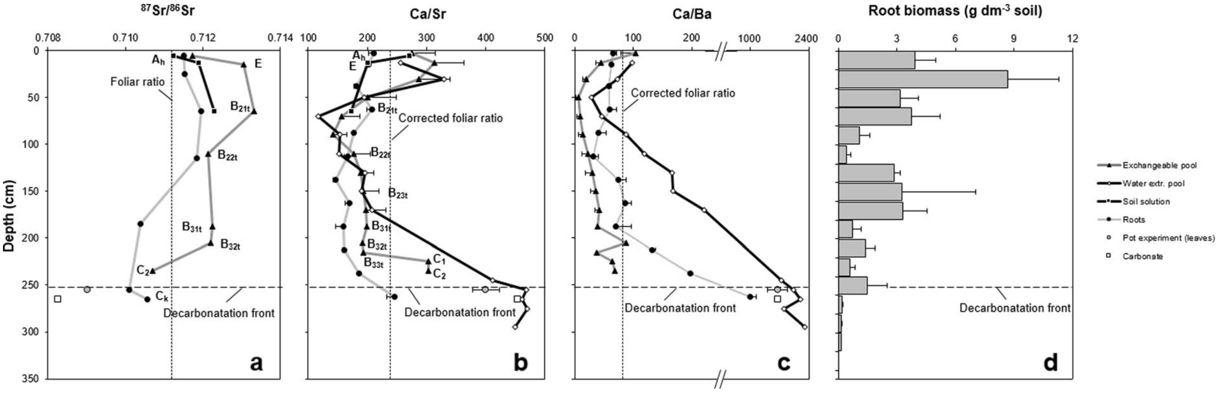


Fig. 3 Evolution with depth of **a** the $^{87}Sr/^{86}Sr$ ratio ($n=1$), **b** the Ca/Sr ratio and **c** the Ca/Ba ratio in the soil exchangeable fraction (*black triangle*) ($n=5$, except for A_h horizon $n=10$), in the soil water extract (*white diamond*) ($n=1$), in the soil solution (*black square*) (data from Drouet et al. 2007b) and in beech roots (*black diamond*) (pooled samples, $n=1$) for a loessic soil profile (MES site). **d** Vertical distribution of root density at the same site. *Error bars* are SD. Carbonate values correspond to the selective dissolution of the C_k horizon

Discussion

Determining the sources of Ca for tree nutrition

In a previous study, we used a mixing equation (Eq. 2) to determine the relative contributions of the two main sources of Ca to a beech stand of the Soignes Regional Forest: mineral weathering release and atmospheric deposition (Drouet et al. 2005a). However, this calculation used only the silicate weathering end-member. The unweathered, calcareous loess layer, which occurs at a depth of 2.5 m, was thought to be beyond the foraging capacity of beech roots. The calculated Ca atmospheric contribution reached on average ~75 %. Here we present clear evidences that the $CaCO_3$ source contributes to the Ca uptake of beech trees. Consequently, the $^{87}Sr/^{86}Sr$ ratio of the mixing compartment (beech leaves) is lowered under the influence of the $CaCO_3$ end-member which has a significantly lower $^{87}Sr/^{86}Sr$ ratio (0.708255) than the silicate end-member (>0.712). Therefore, the contribution of the atmospheric end-member (also characterized by a low Sr isotope ratio: 0.709) was over-estimated in the first study.

The mixing equation for Sr isotopes can be used to determine the relative contributions of only two sources of Ca. Given the depth of root prospecting and the large isotopic gradient from the top soil to the C_k horizon, we used the Ca/Sr ratio as a second independent tracer. The strontium isotopic composition and the Ca/Sr ratio of potential sources of Ca to the trees (i.e., atmospheric precipitation, deep carbonate-bearing layer, and silicate weathering at two depths) were used to draw the mixing diagrams of Fig. 4. As described in the 'Material and Method' section, a discrimination factor has to be used to correct the physiological discrimination process occurring in tree leaves (Dasch et al. 2006; Drouet and Herbauts 2008; Beauregard and Côté 2008; Blum et al. 2012; Lucash et al. 2012). Corrected Ca/Sr ratios of beech leaves were therefore plotted on these diagrams. Exchangeable fractions, soil solutions and root data from both the field and pot experiments (with leaf $^{87}Sr/^{86}Sr$ ratio) were also reported. The position of the roots in such diagrams has to be interpreted with caution because of the uncertainty on the Ca/Sr ratio following the bark stripping treatment before analysis. The dispersion of data for the diverse plant and soil compartments and the absence of distribution along a unique mixing line confirm that the system is influenced by more than two sources. The potential effect of the atmospheric source on the leaf isotopic composition could be quite similar to that of the carbonate source (triangle A vs. B of Fig. 4a). Indeed, $^{87}Sr/^{86}Sr$ and Ca/Sr values of precipitation and carbonate are close (Fig. 4a). However, when precipitation is chosen as end-member (triangle B, Fig. 4a) the decrease of the leaf $^{87}Sr/^{86}Sr$ ratios in some stands (e.g. HUB II) is erroneously attributed to an increase of the atmospheric influence on tree nutrition while this decrease can, more probably be linked to the presence of a carbonate-bearing layer at a rather shallow level in the soil (180 cm depth). This also leads to the unrealistic conclusion that the Ca-poorest sites are the least influenced by atmospheric precipitation. Moreover, quantitative assessment indicates that annual inputs of atmospheric Ca (<10 kg ha^{-1}) are very limited compared to the high soil Ca reserves in the form of carbonate and probably play a minor role in the nutrition of these stands. In addition, the soil exchangeable fraction of the upper meter is marginally influenced by the atmospheric source since mixing calculations excluding the carbonate source indicate that only 20 % (E horizon) and 0 % (B_{21t} horizon) originates from precipitation. Atmospheric inputs of Ca are also low in comparison with the mean annual uptake of Ca by trees which can be estimated at 80 to 90 kg ha^{-1} y^{-1} (mean of a 100-year growth period), from which only 14 to 24 kg ha^{-1} y^{-1} are returned as litterfall and can be recycled. We therefore used the three end-members illuvial B_{21t} and B_{31t} horizons (HCl extracts) and deep carbonate (selective dissolution of the C_k horizon) in triangle A of Fig. 4a. We assumed that the isotopic signature of the B_{21t} horizon was the most representative of the weathering process from 0 to 1 m in depth and that the weathering of the A_h (very thin) and E (low Ca and Sr concentrations) horizons were reduced. In any case, the $^{87}Sr/^{86}Sr$ and Ca/Sr ratios of the HCl extracts for the A_h, E and B_{21t} horizons are relatively well grouped (Fig. 4a). We assessed the isotopic signature of the HCl extract of the B_{31t} horizon as representative of the weathering of the soil layers comprised between one metre and the deeper carbonate-bearing horizon. The choice of these three sources was justified by (1) the large amounts of exchangeable Ca in these horizons (1000 and 7000 kg ha^{-1} for B_{21t} and B_{31t} horizons, respectively, relative to the 200 kg ha^{-1} for the summed A_h and E horizons). The potentially available Ca from the C_k horizon through the dissolution of carbonate can be estimated to 200 t ha^{-1} by slice of 10 cm of this horizon; (2) All the mixing compartments (vegetation, exchangeable fraction and

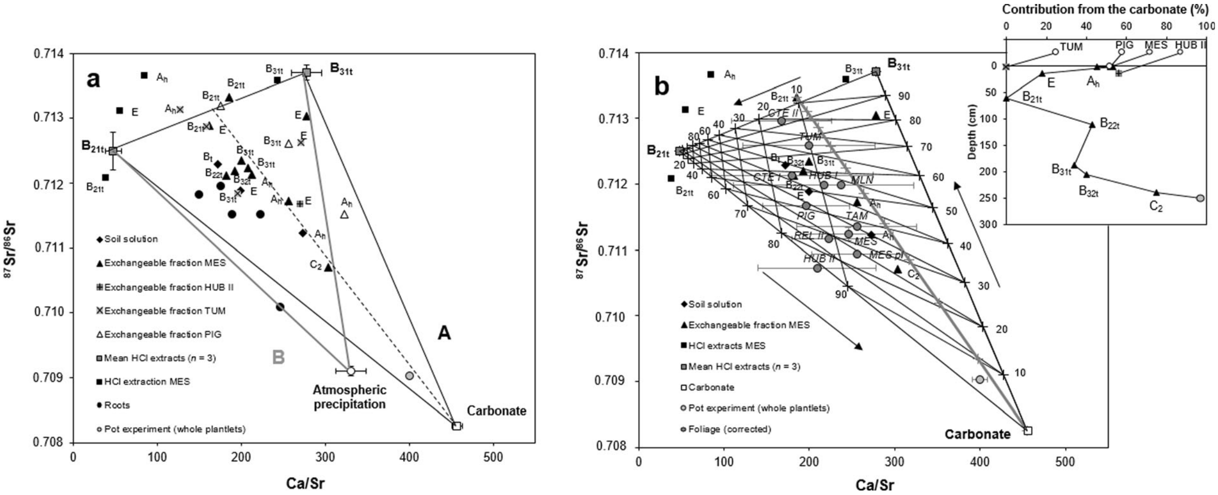


Fig. 4 ^{87}Sr/^{86}Sr ratios as a function of weight Ca/Sr ratios measured in the main compartments of forest ecosystems developed on loess of Central Belgium. **a** The two triangles delimit all the possible compositions of the mixing among 3 end-members: the silicate end-member from 0 to 1 m depth (HCl B_{21t}), the silicate end-member beneath 1 m depth (HCl B_{31t}) and the carbonate from the unweathered loess (>180 cm depth) (triangle A) or the atmospheric precipitation (triangle B). **b** Mixing triangle of the shallow (0–1 m), and deeper (>1 m) silicate end-members and carbonate end-member. Inserted window shows the proportion of Ca inherited from the carbonate source in the exchangeable pool (MES site) and beech leaves of four stands (TUM, PIG, MES and HUB II). *Error bars* correspond to SE. SE of the foliage samples takes into account the error on the DF

soil solution) are circumscribed in the triangle formed by the three poles; (3) most of all, there are highly significant correlations between contribution percentages determined on the basis of the three end-members model and the soil parameters observed independently from this calculation. Indeed, contributions of the carbonate source calculated from this diagram are negatively correlated with the depth of the carbonate-bearing horizon (Fig. 5a). The contribution of the carbonate source increases at the expense of the calcium uptake from the soil layer situated at one metre depth down to the limit with the carbonate-bearing horizon (represented by the HCl extract of the B_{31t} horizon). We observe therefore a strong positive correlation between the proportion of Ca in trees originating from the B_{31t} horizon to the vegetation and the thickness of the layer from one metre depth to the occurrence of the carbonate-bearing horizon (Fig. 5b). Logically, no contribution from the B_{31t} horizon was detected in the HUB II site; this horizon is indeed absent here and replaced by a carbonate-bearing layer starting at the depth of 180 cm and downward. Finally, the contribution from the B_{21t} horizon calculated from this diagram is correlated with the amounts of exchangeable Ca from 0 to 1 m depth (Fig. 5c). The choice of these three end-members is therefore confirmed by parameters which are strictly independent from the calculation of the sources contributions.

Calculations based on these three sources indicate that carbonate is involved in the Ca nutrition of trees in all the sites on loessic material, including the MLN site in which the carbonate-bearing horizon is no longer present (40 % contribution in the MLN site, Fig. 5a, which could correspond to the proportion of Ca inherited from carbonate in the 100–200 cm layer, inserted window of Fig. 4b). This is in line with the view that Ca derived from carbonate dissolution and partially preserved on the exchange complex of the horizons located above the decarbonatation front could be an effective source for tree nutrition. The values of the exchangeable fraction of most horizons are aligned following a line tending to the carbonate end-member and support this explanation (Fig. 4). Horizons deeper than 1 m could be influenced by Ca released by carbonate dissolution whereas topsoil horizons could be influenced by Ca uplift from the C_k horizon eventually returned as litter at the soil surface. Because the Sr isotopic signature of the carbonate and atmospheric precipitation sources are very close, we cannot estimate their respective contributions in the same analysis. However, an estimation of the atmospheric contribution can be achieved using Eq. 2 with data from the TUM site in which the influence of the deep (>300 cm depth) $CaCO_3$ end-member can be considered as minimal. For this TUM site, for which the foliar ^{87}Sr/^{86}Sr ratio is the

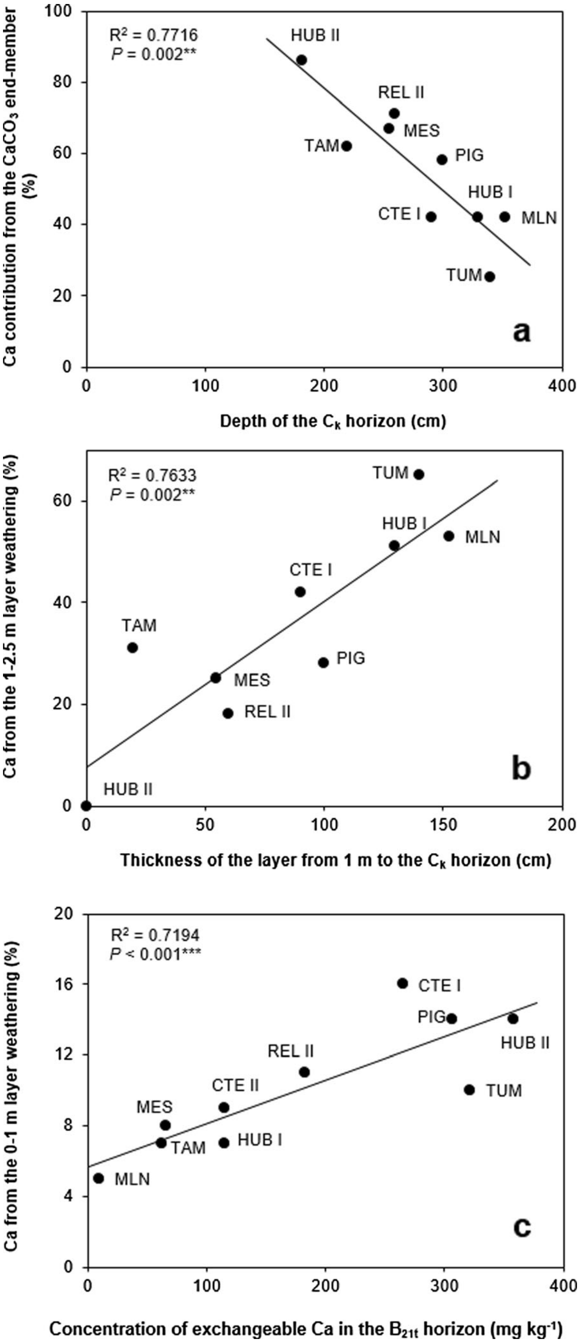


Fig. 5 **a** Relationship between the carbonate source contribution to tree nutrition and the depth of the carbonate-bearing horizon **b** Relationship between the contribution to tree nutrition of the Ca source comprised between 1 m and the variable depth of the carbonate-bearing horizon (>1 m 80) and the thickness of this layer **c** Relationship between the % of calcium in trees from the 0–1 m layer weathering and the content of exchangeable Ca in this layer. The calculations of Ca contributions are always independent to each regressed soil parameter. See text for details

highest of all those measured in the beech stands developed on pure loessic soils, the Ca atmospheric contribution is only 24 %. The influence of atmospheric precipitation is probably even lower than this conservative estimate, given the influence of carbonate on the Sr isotopic signature even when the carbonate-bearing horizon has been totally dissolved.

Influence of the carbonate-bearing horizon

The question that arises from our results is whether the calcareous loess layer has a direct or an indirect influence on the tree mineral nutrition. A direct influence implies that beech roots have access to this C_k horizon despite the fact that it occurs at a depth frequently >250 cm. An indirect influence means that the rooting does not necessary reach this calcareous layer but prospects soil horizons in which the exchangeable pool could contain a significant fraction of Ca inherited from the dissolution of carbonate minerals from the original parent material. In this case, Sr isotopic composition of the exchangeable pool could partly be influenced by the isotopic signature of carbonate. Finally, recycling mechanism through the return of leaf litter which contain Ca partly derived from carbonate (~20 to 85 % following the site) could be considered. Continuous inputs from the deep carbonate via the litter returns could have imprinted a rather unradiogenic Sr isotopic signature in the soil surface layers.

Direct influence

The linear regression of leaf ^{87}Sr/^{86}Sr ratio vs. the depth of the C_k horizon (Fig. 2c and the other regressions on the same figure) which passes at the origin to the value of plantlets cultivated directly on calcareous loess (pot experiment), constitutes the best argument supporting a direct influence of carbonate. This foliar isotopic variation along the soil sequence cannot be explained by any other parameter, given the homogenous soil chemical parameters down to a depth of at least two metres. Moreover, the evolution of the Sr isotopic ratios in the exchangeable fractions is similar down to the B_{31t} horizon in four soil profiles distributed along the soil sequence (MES, REL II, PIG and TUM sites, Drouet et al. 2007b). When stands with and without a deep C_k horizon are separated in two groups, *t*-tests reveal significant differences for a series of element concentrations in leaves: Ca and Mg ($P<0.01$), Sr ($P<0.05$) and Ba

($P<0.05$). The presence of beech roots down to 300 cm in six sites and their vertical distribution measured in the MES site (Fig. 3d) confirm that the carbonate-bearing horizon (>250 cm) is indeed physically reached. The root $^{87}Sr/^{86}Sr$ ratios are higher than that of leaves (0.7112) down to 175–200 cm depth (Fig. 3a). The leaf Sr isotopic signature can therefore only be explained by a large capture of Sr from deep horizons with low $^{87}Sr/^{86}Sr$ ratio, which is necessary to compensate the relative radiogenic uptake in the rest of the soil profile (Fig. 3a).

Indirect influence

Several parameters of the loessic soil indicate that the exchange complex of the horizons that overlie the decarbonatation front has partially preserved Sr and Ca cations released by the dissolution of the carbonate formerly present in the loess. Uptake of cations by tree roots in this mixed exchangeable pool could therefore be considered as influenced indirectly by the carbonate. Calculation of the proportions of the three sources of exchangeable Ca using the mixing diagram (Fig. 4a) shows that Ca inherited from carbonate (C_k horizon) represents a significant proportion of the exchangeable pool (Fig. 4b, inserted window). At about 1 m depth (B_{22t} horizon), the contribution of Ca inherited from the $CaCO_3$ end-member amounts to ~40 %. This proportion does not vary markedly down to 230 cm, but in the underlying C_2 horizon, located just above the decarbonatation front, this contribution increases to 76 %.

The Ca released by the dissolution of carbonate and retained as exchangeable forms could also influence the profiles even if carbonate is now totally dissolved. Accordingly, in the soil developed on deep loess deposit of the MLN site, which is fully decarbonated, the Ca/Sr ratio increases from 200 to 270 cm and, concomitantly, the $^{87}Sr/^{86}Sr$ ratio decreases from 0.721618 in the B_{21t} horizon to 0.711727 in the C-horizon, at a depth of ~285 cm. Resulting contributions of Sr and Ca inherited from the $CaCO_3$ end-member in the exchangeable pool of the C-horizon are 57 and 93 %, respectively (Eqs. 1 and 2). Very high effective base saturation (>90 %) and Ca saturation rates (>60 %) are systematically observed in the deep soil horizons where such a residual cation accumulation has been preserved. The similar evolution with depth of the effective base saturation, Ca/Sr ratio (Fig. S2a) and Ca/Ba ratio (Fig. S2b) in the CTE II and DIEP sites suggests that Tertiary sediments occuring below a loess cover, are also influenced by Sr and Ca cations derived from the dissolution of loess-derived carbonate in the overlying layers. Logically, such trend was not observed in the profile on relatively pure Tertiary material (LND site). In the CTE II and DIEP sites, there is therefore a possibility for trees to prospect Ca-enriched horizons indirectly influenced by carbonate. However, we showed that calcium concentrations in beech leaves are significantly lower in stands without a carbonate layer compared to stands with deep carbonate. Moreover, the calcium status of the stands without C_k horizon is not significantly different from that of the acidophilous stands developed on sandstone in High Belgium (Herbauts 1982). Thus, even if present, this mechanism of uptake of relict Ca dissolved from carbonate and maintained as exchangeable form does not seem to be as effective as the direct influence of carbonate. Indeed, direct influence of carbonate is necessary to maintain the isotopic gradient in soils and is the unique explanation of the gradual variation of foliar Ca concentration in sites along the soil sequence. So, the deep carbonate horizon represents a tremendous source of Ca for vegetation. On the other hand, our results showed that a site with Ca derived at 87 % from the carbonate source displays quite high Ca concentrations in leaves (~8000 $\mu g\ g^{-1}$) but nevertheless somewhat lower compared to situations where carbonate appears at the surface (analogous to pot experiment) or at less than one metre depth in other Belgian stands on calcareous substrates (>11 000 $\mu g\ g^{-1}$; Table 1). Moreover, acidic conditions and low Ca status of the soil surface reflect a moderate annual uptake essentially provided by litter returns influenced by the C_k horizon. The annual input of the carbonate source is probably limited given the low root density in this deep layers. However, long-term moderate but continuous inputs of Ca from this source coupled to a tight cycling of this element are able to maintain contrasting Ca content in litter and to build soil reserves that can reach 60 % of the topsoil horizons. The relatively small annual input of Ca from carbonate coupled with the efficient recycling of this input in the soil surface horizons can reconcile moderate Ca concentrations in leaves and topsoil with a high contribution from the carbonate source reaching 87 % in some sites (HUB II).

Conclusion

This study demonstrates that the Sr isotopic composition of beech trees growing on loessic soils is largely influenced by the root access to a calcareous layer. This layer is located at depths generally greater than 2.5 m and its influence on tree mineral nutrition are direct, when tree roots are able to prospect the C_k horizon, but can also be indirect to a lesser extent, mainly when the development of the rooting zone is restricted to soil horizons where the exchange complex has preserved a significant amount of the Sr and Ca cations formerly released by the dissolution of carbonate minerals. However this last indirect effect cannot be considered to explain the significant isotopic difference between stands developed in soils with or without a deep carbonate-bearing horizon. A direct influence through the small annual uptake of Ca in the C_k horizon coupled with a tight recycling of this element in the top layers is the only mechanism which could explain together 1) the increase of the calculated contribution of carbonate to leaf Ca and the slight increase in litter Ca concentration with the shallow depth of the C_k horizon, 2) the mixing line linking the exchangeable fraction of all the soil horizons to the carbonate source and 3) the relative low foliar Ca content of stands provided at >80 % by the carbonate source (e.g., HUB II site). The input of Ca from atmospheric precipitation estimated in a site with no direct carbonate influence can be estimated to a maximum of 24 %.

The access of beech roots to a deep carbonate source has an important ecological implication in regard with the acid depositions combined with intensive logging in this forest. In the absence of a deep carbonate source, the tree nutrition status of these forest stands could be more critical. This study shows that the use of a soil sequence within a homogeneous substrate is essential to identify and quantify the influence of more than two sources of nutrient in complex systems.

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