Crop Fertility Conditions in North-Eastern Gaul During the La Tène and Roman Periods: A Combined Stable Isotope Analysis of Archaeobotanical and Archaeozoological Remains

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Introduction

Domestic crops originating from the Near East were introduced into Europe at the turn of the 7th millennium cal BC. Along their western and northern diffusion across Europe, crops had to be acclimatised to a great variety of climatic and environmental conditions – including topography and soil – and cultivation practices had to adapt in order to improve and maintain yield and productivity (Bakels 1997; Araus et al. 2014). From the earliest steps of agriculture, the conditions of cultivation were managed and improved through irrigation and fertilisation practices, as evidenced from archaeobotanical and palaeosols studies (Simpson et al. 1997; Guttmann, Simpson, and Davidson 2005; Meharg et al. 2006), nitrogen isotope analysis of charred cereal grains (Araus, Febrero, et al. 1997, 2014; Riehl et al. 2014). Manuring practices were investigated on the basis of palaeosols studies (Simpson et al. 1997; Guttmann, Simpson, and Davidson 2005; Meharg et al. 2006), nitrogen isotope analysis of charred cereal grains (Bogaard et al. 2007; 2013; Aguilera et al. 2008; Araus et al. 2014), and archaeobotanical weed assemblages (Jones et al. 2000; Charles et al. 2003; Bogaard et al. 2007).

Later on, plant cultivation had to face other challenges including climatic fluctuations at multi-century scales, and socio-economical evolutions. Among those, the urbanisation leads to a profound reorganisation of the rural landscapes in Western Europe. During the Late Iron Age indeed, the densification of settlements in Northern France implied an important extension of the land surfaces associated with crop production (Malrain et al. 2015) but this went also with a more extensive management of cereals cultivation (Zech-Matterne and Brun 2016). During the Roman period, specialisation in crop production involved massively the naked wheats (see below).

The aim of this paper is to explore how fertilisation middles helped Gallic and Roman farmers to face new challenges in crop production when the urbanisation of Northern Gaul started in the 2nd century BC and a large feeding trade-system was established in the decades following the Roman conquest.

Crop production in the Roman and La Tène period

Cereal cultivation in north-eastern France has been investigated by numerous bioarchaeological studies.
This has enabled trends in the evolution of cultivation practices, and the intentional selection of plant species from the Middle Iron Age to the end of the Roman period to be described. Analyses of the plant remains, from 2200 contexts and 170 occupations dated from the 4th century BC to the 5th century AD, highlighted specialised crop cultivation, focused primarily on the large-scale exploitation of emmer and hulled barley, associated with spelt wheat in some locations (Zech-Matterne, Wiethold, and Pradat 2014). In France, the evolution of the topographic location of rural settlements during the 2nd century BC (La Tène B); at a time when plateaus started to be assigned to agricultural activities (Malrain, Blancquaert, and Lorho 2013). During the 2nd century BC, the emergence of oppida and small towns established a new framework for the control of production and food supply. This incipient urbanisation developed a growing need for cereals that were free-threshing and ready to be milled or consumed. Naked wheat began to replace emmer and spelt, which was much harder to dehusk and which returned lower yields. The cultivation of bread wheat rose progressively from the end of the La Tène period (Zech-Matterne, Wiethold, and Pradat 2014). The Roman conquest in 57–52 BC accelerated this new requirement, and bread wheat cultivation was established at a regional scale in the Seine, Oise and Aisne river valleys. In the 1st century AD, the zoning of areas, which persisted throughout the Roman period, was initiated: a northern zone, in which hulled wheat was maintained; a central zone, where naked wheat was intensively cultivated; and an eastern zone, where barley was the dominant crop, even though naked wheat stocks were being traded from southern regions. The strong association between crops and animal breeding species at a regional scale has already been highlighted (Lepetz and Matterne 2003): in the northern regions (Nord-Pas-de-Calais and Picardy) the prevalence of hulled wheat was associated with cattle breeding; while in the southern regions (Île-de-France) the presence of naked wheat was associated with caprines. From the 2nd century AD onwards, pulse cultivation began to increase in importance in areas where naked wheat had previously been preferable; and in the northern part of the ‘naked wheat area’, spelt wheat started to be more heavily exploited. The limit between these two zones fluctuated through time. Spelt and bread wheat are both suitable for bread making and were probably interchangeable in terms of consumption. However, spelt is much less demanding in terms of manure and soil tillage, whereas bread wheat, though more productive, is much more demanding in terms of soil requirements (Campbell 1997).

Soil exhaustion and adopted solutions

Naked wheat was cultivated in the Île-de-France region during all five centuries of the Roman period. This may have caused a progressive depletion of soil nutrients, though most of the Paris Basin plateaus are covered with thick layers of loess and loamy soils of the ‘brown’ and ‘washed brown’ types (luvisols). Soil exhaustion may be reflected by the implementation of crop rotation, including leguminous plants, a century after the generalisation of naked wheat cultivation (Zech-Matterne, Wiethold, and Pradat 2014). But were these Roman solutions, such as the use of manure, green manure and the introduction of rotation cycles involving pulses, effective? A tentative approach to answer this question was previously carried out using the functional ecology of the weed communities of cultivated fields (Zech-Matterne and Brun 2016). The composition of weed assemblages is responsive to major changes in cultivation practices, including manuring (Jones et al. 2000; Charles et al. 2003; Bogaard et al. 2007). A large-scale statistical analysis (i.e. 96 sites and 119 weed species) highlighted that in 6th and 5th century BC rural settlements, the dominant weed species reflected cultivation on the most fertile plots; partly because many farmsteads were established on alluvial soils and fields were regularly manured. On the contrary, during the Late La Tène period all types of soil were exploited, but from the beginning of the Roman period, middle quality soils prevailed and poor soils were as well cultivated. This could indicate a diversification of farming land due to constraints on arable land access, or a lack of fertilisation on some plots owing to the establishment of extensive systems (Zech-Matterne and Brun 2016).

Therefore, the objective of this study was to elucidate if the specialisation of agricultural species and cultural practices in northern Gaul, during the Middle La Tène period and the beginning of the Roman period, depended on soil impoverishment over time, especially where intensive and specialised bread wheat cultivation had challenged yield maintenance. A particular focus was applied to determine how the Romans were able to manage soil fertility on the chalky plains of the Champagne region, where strong edaphic constraints existed (deficiencies of nutrients and water), and to evaluate the role of animal manuring practices in the management of soil fertility. To achieve these objectives, stable isotope analysis was performed on archaeobotanical (carbonised cereal grains) and archaeozoological remains (animal bones).

Stable isotope background

The stable carbon ($\delta^{13}$C) and nitrogen isotope ratios ($\delta^{15}$N) in plants are related to environmental
parameters and physiology (Farquhar, Ehleringer, and Hubick 1989; Araus et al. 2003; Ferrio et al. 2003). A growing number of studies apply this approach to archaeological plant remains in order to reconstruct past climatic and agricultural conditions (Araus, Bort, et al. 1997; Bogaard et al. 2007, 2013; Aguilera et al. 2008, 2012; Araus et al. 2014; Riehl et al. 2014).

Stable carbon isotopes in plants

The C₃ photosynthetic pathway predominated European plants (Pyankov et al. 2010), including cultivated cereals, at least until the Early Bronze Age. During this time, millet, a C₄ cereal, appears for the first time in north-eastern France (Toulemonde 2013); however, the low proportion or absence of millet in the studied sites from the La Tène period does not suggest large-scale cultivation (Jacob, Disnar, and Bardoux 2008; Zech-Matterne et al. 2009).

δ¹³C of plants is isotopically depleted in ¹³C respect to the atmospheric CO₂, which is the carbon source for photosynthesis (Farquhar, Ehleringer, and Hubick 1989). Environmental factors, including temperature, precipitation, irradiance and vapour pressure deficit do exert an influence on the CO₂ interchange between plants and atmosphere, and consequently impact the δ¹³C values of C₃ plants. The plant reacts under environmental stress (i.e. low light intensity or low water availability) by closing the stomata, leading to an increase of δ¹³C values (Farquhar, Ehleringer, and Hubick 1989). Inversely, when the environmental conditions are favourable, the stomata remain open and the Rubisco enzyme discriminates against the ¹³C, causing an increase in negative δ¹³C values (Condon, Richards, and Farquhar 1992; Araus, Bort, et al. 1997; Ferrio et al. 2003). Consequently, in dry environments, comparably low δ¹³C values in cultivated plant remains may highlight irrigation practices (Araus et al. 2003; Ferrio et al. 2005).

Stable nitrogen isotopes in plants

Non-leguminous plants synthesise proteins from nitrogen (N) absorbed in the soil. Plants need microorganisms to transform N from organic material into a soluble form, in order to absorb it through the roots as nitrates (NO₃⁻) and ammonium (NH₄⁺), causing different N isotope compositions (Robinson 2001). Differences in nitrogen sources, patterns of nitrogen uptake and/or assimilation pathways can lead to different discrimination rates against ¹⁵N (Evans 2001). The natural abundance of stable N isotopes in soils and plants is affected by abiotic factors including temperature and precipitation regimes. These induce differences in nitrogen cycling (Handley et al. 1999; Amundson et al. 2003; Aranibar et al. 2004) or soil processes, including biotic factors such as land use and agricultural practices (Comimso and Nelson 2006; Bogaard et al. 2007; Compton, Hooker, and Perakis 2007). All these considerations exercise influence on the isotopic fractionations during the soil-plant-animal interactions, and are entailed in the complex interpretation of the isotopic signal of the N cycle: the δ¹⁵N values of plants provide information about the δ¹⁵N values of the different assimilated N forms, the relationship between plant N demand and N supply and the rate of N derived from the organic material decomposition (Evans 2001; Aguilera et al. 2008; Kalc-sits, Buschhaus, and Guy 2014). Despite this, the δ¹⁵N of plants in natural environments can be considered to be a reliable approximation on the δ¹⁵N of the environmental substrate (Handley and Raven 1992; Dawson et al. 2002; Marshall, Brooks, and Lajtha 2007; Bai and Houlton 2009).

The influence of organic fertilisers, specifically those originating from animal dung, on soil δ¹⁵N values has been demonstrated in long-term agricultural experiments in temperate Europe (Riga, Praag, and Brigode 1971; Gerzabek, Haberhauer, and Kirchmann 2001; Bol et al. 2005): the δ¹⁵N values of cereals was increased (Bol et al. 2005; Fraser et al. 2011). In addition, the enrichment of the δ¹⁵N values of cereals consecutive to N inputs through animal manuring is related to the intensity and duration of manuring (Bol et al. 2005; Choi et al. 2006; Bogaard et al. 2007; Szpak et al. 2012). In particular, some long-term experiments were carried out in temperate zones: Rothamsted (UK), Askov (Denmark) and Bad Lauchstädt (Germany), in which the manuring impact on the δ¹⁵N values of cereals was assessed (Bol et al. 2005; Bogaard et al. 2007; Fraser et al. 2011). In these studies, a direct connection was established between fertiliser application and the enrichment of the cereals δ¹⁵N values, recording differences from 4 to 9‰ between unmanured and manured plots, depending of studied sites (Bogaard et al. 2007; Fraser et al. 2011). In summary, different ranges of δ¹⁵N values were identified for modern cereals grown under a gradient of intensity of fertilisation practices: δ¹⁵N values below 2.5‰ mainly corresponded to unmanured fields (Bol et al. 2005; Fraser et al. 2011); δ¹⁵N values from 2.5 to 6‰ reflected a medium level of fertility resulting from light manuring, a residual effect after a period of heavy manuring, or the natural fertility in the first years of a newly cultivated land (Fraser et al. 2011); while δ¹⁵N values above 6‰ suggested intensive and systematic manuring (Fraser et al. 2011).

Depending on the climate, soil type and history of use, these threshold values may vary at a local scale. One particular challenge is to determine the baseline δ¹⁵N value of unmanured soil. In this case, the weed flora associated with the grain assemblage could not be used as a reference for the baseline nutritional status of the soil. Indeed, its presence within the crop assemblage suggests it was grown in the same fields under the
same manuring regime. However, an estimation of the $\delta^{15}$N values of non-fertilised plants was able to be obtained indirectly from the bone collagen $\delta^{15}$N values of associated wild herbivores, taking into account a 3–4‰ $\delta^{15}$N-enrichment between diet and bone collagen (Schoeninger and DeNiro 1984; Bocherens and Drucker 2003).

The interpretation of the $\delta^{15}$N values of archaeological plants was able to be used both as an integrative proxy to characterise local nitrogen cycling processes, and as an indicator of the nutritional status of ancient crops (Handley and Raven 1992; Amundson et al. 2003; Aguilera et al. 2008; Bai and Houlton 2009).

Methodological aspects

The majority of cereal grains retrieved from archaeological sites was preserved in a charred state. Various experiments carried out at different temperatures, times and atmospheric conditions regarding the possible effect of carbonisation on the isotopic signal of cereal grains, have so far produced divergent conclusions (Marino and DeNiro 1987; Araus, Febrero, et al. 1997; Bogaard et al. 2007; Ferrio et al. 2007; Aguilera et al. 2008; Fraser et al. 2013; Nitsch, Charles, and Bogaard 2015); but at the moment, the general consensus is that it has either no impact (Marino and DeNiro 1987; Araus and Buxó 1993; Kanstrup et al. 2012; Fraser et al. 2013) or a minimum impact (Nitsch, Charles, and Bogaard 2015) on the stable carbon isotope composition. As for the effect of carbonisation on the nitrogen isotope composition, there is contradictory evidence either for no signal modification (Bogaard et al. 2007; Aguilera et al. 2008; Kanstrup et al. 2012) and modification leading to a significant $\delta^{15}$N-enrichment (Fraser et al. 2013; Styring et al. 2013; Nitsch, Charles, and Bogaard 2015).

The chemical pre-treatment of archaeobotanical material prior to analysis is another controversial issue. This pre-treatment is intended to remove postdepositional contamination from the sediment, which could alter the stable isotope ratios measured in the grains. Those contaminants potentially include carbonates, nitrates and/or humic acids, depending on the soil composition and soil conditions where the archaeological seeds were preserved. The necessity of a pre-treatment, and the choice between several alternatives (acid/ base concentrations, soaking times and temperatures: DeNiro and Hastorf 1985; Bogaard et al. 2007; Fraser et al. 2013; Vaiglova et al. 2014) depends on the soil conditions. Some studies have demonstrated no difference between pre-treated and non-treated archaeological samples (Lightfoot and Stevens 2012; Wallace et al. 2015). Among the pre-treatments currently undertaken, the most commonly applied involves a 1 or 6 M HCl acidification (DeNiro and Hastorf 1985; Fiorentino et al. 2008; Brock et al. 2010; Kanstrup et al. 2012). In this study, we also tested to see whether different concentrations of these would lead to significantly different results.

Materials and methods

Archaeobotanical remains

The charred grains came from 12 archaeological sites comprising 19 occupation periods situated in the Île-de-France and Champagne regions, dating from the Late La Tène period to Late Antiquity (Figure 1 and Table S1). All these sites can be considered as farmsteads, of the ferme indigènes (enclosures), small Roman farms or villa rustica type, with the exception of Acy-Romance, a ‘village’ and Jouars-Ponchartrain, a small town. The samples came from deeply or semi-excavated structures devoted to grain storage (mainly storage pits and cellars) or from rubbish pits and rubbish deposits in the enclosure ditches. Most of the assemblages can be regarded as ‘mass finds’ and their composition is rather homogenous. Crop management appears mainly to have been based on cereals (i.e. hulled and naked wheat, hulled barley), alternating with fallow, indicated by the composition of the arable weed spectra specific for ancient cultivated or untilled places, as Artemisieta. Diachronic trends were able to be explored at Palaiseau and Epiays-lès-Louvre, where between three and five archaeological phases were represented.

Both regions strongly differ in the types of prevailing soils. In the Île-de-France region, 10 archaeological sites were studied on the loamy plateaus of the northern part of the Paris Basin; an area still regarded as a major cereal production basin, dominated by the cultivation of bread wheat. The calcareous substratum is covered by several metres of very fertile wind silt (loess). These luvisol soils have a high capacity for water retention, but exhaustible nutrient resources and calcium content. Emmer and hulled barley were the dominant crops during the Early Protohistoric period, but were replaced by naked wheat from the second half of the 2nd century BC (Zech-Matterne, Wiethold, and Pradat 2014).

Two archaeological sites were selected in the Champagne-Ardenne region, more specifically on the plaine crayeuse (chalky plain). Here the chalky substratum is directly covered by thin layers of chalk nodules resulting from its disintegration, known locally as graves-chênes. The shallow rendzina soil contains active limestone, which can generate a risk of ferric chlorosis. Due to the unavailability of iron, the constitution of chlorophyll is disrupted, as is the photosynthesis. The hydric reserves of the superficial layers are also lowered by the number of micro-fractures in the chalk. The most frequently cultivated cereals were hulled barley and spelt (Zech-Matterne, Wiethold, and Pradat 2014).
2014). A total number of 148 sets of charred cereal grains were analysed, belonging to four species: *Triticum aestivum* – bread wheat and *Hordeum vulgare* – barley (free-threshing cereals); and *Triticum spelta* – spelt and *Triticum dicoccum* – emmer (hulled cereals). Each sample set included 10 charred grains from the same context and stratigraphic unit. All the crops sampled derived from defined concentrations and storage structures (Table S1).

**Pre-treatment experiment**

The experiment involved 795 archaeological cereal grains. The four species (spelt, barley, emmer wheat, bread wheat) were each represented by approximately 200 grains. Each species came from a single archaeological context, but the different species came from different sites all located in North-Eastern France. Spelt came from Amiens ‘ZAC Cathédrale’, barley came from Chambly ‘La-Marnière’; emmer and bread wheat came from Mareuil-lès-Meaux ‘La Grange du Mont’ (Figure 1). At all sites loamy soils with a small proportion of organic matter prevailed, so no humic acid contamination was expected (Vaiglova et al. 2014). Therefore, the pre-treatment only involved the first acid step; further basic acid steps were not applied (DeNiro and Hastorf 1985; Aguilera et al. 2008).

Two HCl concentrations (1 and 6 M) were tested on entire and powdered grains. The grains were treated individually with HCl during 24 h at room temperature, soaked in distilled water three times (24 h-12 h-6 h), oven-dried at 60°C for 48 h, milled to a fine powder (only entire grains) and homogenised. A total of 80 bulk samples were analysed (5 grain samples*4 pre-treatments*4 cereals), each of which was comprised of 10 grains (with the exception of two groups with 7 and 8 grains) to minimise the effect of inter-grain variability (Figure S1).

**Archaeozoological remains**

The reference δ¹⁵N value for unmanured soils was estimated from the analysis of local wild herbivore bone collagen (*Cervus elaphus*: red deer) (cf. Bogaard et al. 2013). Although domestic animals largely predominate the faunal assemblages from the sites, they were avoided for this purpose because of the observed association between cultivation and husbandry in these agricultural systems (Lepetz and Matterne 2003): domestic animals may have been fed the by-products from cereal cultivation. From the assemblages of Palaiseau, Jouars-Pontchartrain and Acy-Romance (Table 2), 30 specimens were selected.

To commence collagen extraction, 300 mg of powdered bone was used following the procedure described in Bocherens et al. (1991). The δ¹³C and δ¹⁵N values of the red deer diet were estimated by applying a 5‰ ¹³C-enrichment (Lee-Thorp 1989; Ambrose and Norr 1993) and a 3‰ ¹⁵N-enrichment between diet and bone collagen (Schoeninger and DeNiro 1984; Bocherens and Drucker 2003).

**Stable isotope analysis**

Aliquots of 1 mg for archaeological cereal grains and 500 μg for bone collagen were weighed into tin capsules for coupled δ¹³C and δ¹⁵N measurements. The capsules were combusted in an Elemental Analyzer Thermo
Flash 2000 interfaced to a Thermo DeltaV Advantage isotope ratio mass spectrometer. Isotope ratios are expressed as per mille deviations using the δ notation relative to the air N₂ and VPDB standards, for δ¹⁵N and δ¹³C, respectively. The analytical precision (standard deviation of working standards) determined for all runs was 0.16‰ for δ¹⁵N and 0.15‰ for δ¹³C.

In order to compare the δ¹³C values from different periods, the carbon isotope discrimination of archaeological grains (Δ¹³C) was calculated following the equation of Farquhar, Ehleringer, and Hubick (1989); in order to correct for fluctuations in the δ¹³C in atmospheric CO₂ throughout the Holocene (Ferrio et al. 2007) (Table 1).

In addition, stable isotopes were used as direct sources of information to reconstruct environmental conditions without any correction for carbonisation effect. A recent study by Nitsch, Charles, and Bogaard (2015) in which cereals and pulses were considered together, recommends applying a 0.31‰ correction to the δ¹⁵N values of charred remains, even though charring caused a bigger shift on pulses than cereals. Given the uncertainties on the existence of any systematic effect of carbonisation on the δ¹³C and δ¹⁵N of cereals grains, and given that when observed the shifts are of similar amplitude as the analytical precision of IRMS, we decided to apply no correction in this study.

### Statistical analyses

All data were subjected to analysis of variance (ANOVA) to ascertain the effect of chemical treatments. Unless otherwise stated, differences were considered statistically significant when \( P < 0.05 \). All analyses were carried out using standard SAS-STAT procedures.

### Results

#### Pre-treatment experiment

A three-way ANOVA was conducted on 80 sample sets to examine the main effects of species/context, concentration, the state of the grain (i.e. powdered or entire) and the interaction between different pre-treatments and species/context on the stable isotope values of archaeological grains (Supplementary Information Table S2). For δ¹⁵N values, species/context and the state of the grain yielded statically significant values at the 0.05 significance level, but no significant difference (NS, \( P = 0.32 \)) existed among 1 and 6 M HCl concentrations. A small and significant difference (0.191‰, \( P = 0.022 \)) existed with regards to the state of the grain during pre-treatment: the chemical pre-treatment carried out on entire grain yielded higher δ¹⁵N values (Figure 2). The interaction effects of the factors were not significant.

Regarding the δ¹³C values, no significant difference (NS, \( P = 0.881 \)) between the state of the grain during chemical pre-treatment was found; however, the HCl concentrations did influence the carbon isotopic signal (\( P = 0.033 \)). The cereal grains treated with the lowest concentration of HCl (1 M) presented a mean value of −23.17‰, while the cereals grains treated

### Table 1. Stable isotopes results of cereals grains analysed for each species and the chronological data of the archaeological sites.

| Id       | Site            | Species                  | Date       | \( \delta^{13}C_{air} \) | n⁰ | \( \delta^{13}C \) (mean ± 1σ) | \( \delta^{15}N \) (mean ± 1σ) |
|----------|-----------------|--------------------------|------------|--------------------------|----|-------------------------------|-------------------------------|
| 1        | ILE-DE-FRANCE   | Hordeum vulgare          | 600–400 BC | −6.48                    | 5  | −24.5 ± 0.29                 | 4.4 ± 0.46                   |
| 2        | BAILLY          | Triticum aestivum        | 600–400 BC | −6.48                    | 5  | −23.7 ± 0.33                 | 3.3 ± 0.4                    |
| 3        | BAILLY          | Triticum aestivum        | 150–90 BC  | −6.44                    | 5  | −23.9 ± 0.24                 | 6.5 ± 0.32                   |
| 4        | PALAISEAU       | Triticum aestivum        | 30 BC–30 AD| −6.41                    | 5  | −23.8 ± 0.26                 | 6.6 ± 0.31                   |
| 5        | PALAISEAU       | Triticum aestivum        | 100–200 AD | −6.38                    | 5  | −23.8 ± 0.19                 | 6.8 ± 0.14                   |
| 6        | VARENNES-SUR-SEINE | Triticum aestivum     | 120–60 BC  | −6.43                    | 5  | −24.3 ± 0.16                 | 4.8 ± 0.38                   |
| 7        | ROISSY          | Triticum aestivum        | 60–30 BC   | −6.42                    | 5  | −23.9 ± 0.28                 | 3.0 ± 0.62                   |
| 8        | BONNEUIL        | Triticum aestivum        | 0–100 AD   | −6.4                    | 5  | −22.2 ± 0.21                 | 0.7 ± 0.48                   |
| 9        | MARIEUL-LES-MEAUX | Hordeum vulgare        | 0–200 AD   | −6.4                    | 3  | −23.7 ± 0.12                 | 4.6 ± 0.47                   |
| 10       | MARIEUL-LES-MEAUX | Triticum aestivum      | 0–200 AD   | −6.4                    | 20 | −22.2 ± 0.23                 | 5.4 ± 0.19                   |
| 11       | HOUĐAN          | Triticum aestivum        | 200–300 AD | −6.36                    | 2  | −24.0 ± 0.14                 | 2.7 ± 0.15                   |
| 12       | HOUDAN          | Triticum aestivum        | 200–300 AD | −6.36                    | 5  | −23.0 ± 0.42                 | 5.8 ± 0.97                   |
| 13       | EPIAIS-LÈS-LOUVRES | Triticum aestivum     | 30 BC–0 AD  | −6.41                    | 5  | −21.9 ± 0.15                 | 3.6 ± 0.34                   |
| 14       | EPIAIS-LÈS-LOUVRES | Triticum aestivum      | 50–150 AD  | −6.39                    | 3  | −23.8 ± 0.09                 | 5.1 ± 0.2                    |
| 15       | EPIAIS-LÈS-LOUVRES | Triticum aestivum      | 250–350 AD | −6.35                    | 5  | −22.4 ± 0.31                 | 3.0 ± 0.51                   |
| 16       | EPIAIS-LÈS-LOUVRES | Triticum aestivum      | 300–350 AD | −6.35                    | 5  | −23.3 ± 0.15                 | 5.1 ± 0.27                   |
| 17       | EPIAIS-LÈS-LOUVRES | Triticum aestivum      | 300–400 AD | −6.35                    | 5  | −23.3 ± 0.21                 | 4.8 ± 0.41                   |
| 18       | JOUARS-PONTCHARTRAIN | Hordeum vulgare  | 30 BC–15 AD | −6.41                    | 5  | −23.8 ± 0.24                 | 8.7 ± 0.7                    |
| 19       | JOUARS-PONTCHARTRAIN | Triticum aestivum     | 30 BC–15 AD | −6.41                    | 2  | −23.0 ± 0.22                 | 8.2 ± 0.2                    |
| 20       | MORGINCHAMPIGNY  | Triticum aestivum        | 325–250 BC | −6.46                    | 5  | −23.3 ± 0.12                 | 4.6 ± 0.38                   |
| 21       | MORGINCHAMPIGNY  | Triticum spelta          | 325–150 BC | −6.46                    | 3  | −22.5 ± 0.32                 | 4.7 ± 0.06                   |
| 22       | ACY-ROMANCE     | Hordeum vulgare          | 150–100 BC | −6.45                    | 5  | −23.8 ± 0.1                   | 1.3 ± 0.19                   |
| 23       | ACY-ROMANCE     | Triticum spelta          | 150–100 BC | −6.45                    | 5  | −22.4 ± 0.29                 | 2.9 ± 0.42                   |
| 24       | CHAMPFLEURY     | Triticum spelta          | 60–30 BC   | −6.42                    | 5  | −22.7 ± 0.29                 | 4.3 ± 0.25                   |

\( \delta^{13}C \) in atmospheric CO₂ (Ferrio et al. 2005).

n⁰ is the number of analysed aliquots. Each aliquot includes 10 grains.
with a strong acid (6 M HCl) presented the slightly more positive mean value of ~23.06‰. For C isotopes, the interactions were also not statistically significant.

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**\( \delta^{15}N \) and \( \delta^{13}C \) of archaeological crop seeds**

The \( \delta^{15}N \) values of charred cereal grains presented a wide range among sites, periods and species (Table 1, Supplementary Information Table S3). The values measured in the Champagne soils were within the range of those measured in the Ile-de-France: varying between 0.73‰ at Bonneuil and 8.71‰ at Jouars-Pontchartrain (Figure 3(a,b)). Significant interspecific differences appeared between crops grown at the same site: Bailly (1), Mareuil-lès-Meaux (6), Houdan (7) and Acy-Romance (11). The \( \delta^{15}N \) values of *H. vulgare* differed significantly from the *Triticum* species, though not always in the same direction, with higher \( \delta^{15}N \) values for *H. vulgare* compared to *Triticum* at Bailly (1) and lower values in all other instances.

On a regional scale, no temporal trends were observed. At Palaiseau (2) and Epiais-lès-Louvres (8), where the sampling included various occupation phases, different temporal patterns were observed. No significant variations at Palaiseau (\( \delta^{15}N = 6.64 \pm 0.28\%o \)), during a period of nearly 300 years, were noted; but fluctuations over a range of \( \delta^{15}N \) values (from 2.56 to 5.35 %o) were detected at Epiais-lès-Louvres, during a period of 350 years.

The \( \Delta^{13}C \) values varied markedly among species, sites and across time periods. The values of both regions were comprised between 15.87 %o (*T. aestivum* of Epiais-lès-Louvres, earliest phase) and 18.46 %o (*H. vulgare* at Bailly). The mean specific \( \Delta^{13}C \) values were 17.97 ± 0.36%, 17.07 ± 0.82%, 17.14 ± 0.24% and 16.46 ± 0.32% for *H. vulgare*, *T. aestivum*, *T. dicoccum* and *T. spelta*, respectively. Interspecific differences were observed between crop species cultivated at the same site. Barley delivered higher \( \Delta^{13}C \) values than wheat (*T. aestivum*, *T. dicoccum* and *T. spelta*) in all instances, with a maximum difference of 1.6‰ between barley and bread wheat at Mareuil-lès-Meaux (6). On the time scale, the \( \Delta^{13}C \) values of *T. aestivum* remained stable (17.85 ± 0.22‰) throughout the temporal sequence at Palaiseau (2); while fluctuations over a range of 2‰ were visible throughout the occupation at Epiais-lès-Louvres (8), following the same directions as those observed in the \( \delta^{15}N \) values.

**Bone collagen \( \delta^{15}N \) and \( \delta^{13}C \)**

Results from the stable isotope analysis of the red deer bone collagen are reported in Table 2 and Figure 4. The C (34.7–43.2%) and N contents (12.6–15.7%) and the C:N ratios, comprised between 3.1 and 3.3, allowed us to consider all extracts reliable for interpretation (DeNiro and Hastorf 1985).

Intersite differences in the red deer \( \delta^{13}C \) values were not significant. The mean \( \delta^{13}C \) value for all sites was -21.9 ± 0.46‰. Significant differences in the red deer \( \delta^{15}N \) values were observed between sites (\( F = 7.73, P = 0.002 \)). Palaiseau delivered the highest \( \delta^{15}N \) values (5.7 ± 0.69‰), while Acy-Romance presented the widest variability (4.9 ± 0.91‰).

The \( \delta^{15}N \) values for the red deer’s diet (‘wild plants’) are reported in Figure 4; by comparison, the cultivated cereals had higher \( \delta^{13}C \) values (by approximately 3–4‰). The estimated \( \delta^{15}N \) values for wild plants were 2.7 ± 0.7, 1.3 ± 0.84 and 1.8 ± 0.9‰ at Palaiseau, Jouars-Pontchartrain and Acy-Romance, respectively.

The \( \delta^{15}N \) values measured in cereals are also considerably higher than those estimated for wild plants at Palaiseau and Jouars-Ponchartrain, but are comparable to those estimated for the wild plants at Acy-Romance.

**Discussion**

**Pre-treatment for isotopic analyses of archaeological grains**

Results from the experimental pre-treatments observed no clear pattern regarding the effect of the HCl concentration or the state of the grain on stable isotope values.

Changes in acid concentration did not imply any difference to the \( \delta^{15}N \) values, contrary to the results of Vaiglova et al. (2014). A small but significant difference in the \( \delta^{15}N \) values between the state of the grain during the pre-treatment was detected (0.19‰), although this is close to the IRMS sensitivity value; but the possibility of losing a high percentage of the material due to milling, prior to pre-treatment, and the requirement of the use of a centrifuge at each step was not justifiable for such a negligible difference.

Conversely, although the \( \delta^{13}C \) samples treated with 1 or 6 M HCl differed significantly, this difference was too small (0.11‰), again, close to the IRMS sensitivity) to be considered important for the interpretation of the crop stable isotope results. The fact that a high acid concentration could modify the carbon isotopic signal has already been reported by Vaiglova et al. (2014). This study compared treated (with gentle or harsh acid) and untreated samples of legumes and cereals which were then analysed together. The impact of the harsh acid concentration on the \( \delta^{13}C \) values was greater, but this could be attributed to the fact that a distinct composition exists among samples of legumes and cereals (López et al. 2005).

We can deduce no significant effect of using pre-treatments to remove contamination from entire or
powdered grains. Consequently, for this study the entire grains were treated with 1 M HCl in order to minimise material loss and to apply less aggressive methods prior to stable isotope analyses.

Environmental conditions from the Δ$^{13}$C of archaeological cereal grains

Most studies examining the effects of environmental factors on plant stable carbon isotopic composition have focused on arid or semi-arid climates where water availability applies major restrictions on plant growth (Araus et al. 2003; Ferrio et al. 2005; Flohr, Jenkins, and Müldner 2011; Riehl et al. 2014). In temperate zones, where water availability is not a limiting factor for plant growth, the relationship between the Δ$^{13}$C and water conditions is not clearly defined, since other factors like irradiance or temperature can exercise influence on photosynthesis (Khazaei et al. 2008). In any case, many studies have shown correlations between δ$^{13}$C or Δ$^{13}$C values and amount of precipitation or irrigation, but those may also differ with the crop growing season. While some studies correlated the δ$^{13}$C with the total water inputs (i.e. precipitation and irrigation water over the whole growing season) (Flohr, Jenkins, and Müldner 2011; Wallace et al. 2013); others highlighted good correlations between δ$^{13}$C values and total water inputs during grain filling (i.e. precipitation plus irrigation water from flowering to maturity stage) (Araus, Febrero, et al. 1997; Araus et al. 1999; Ferrio et al. 2005). In the present study, a qualitative reconstruction was

Figure 2. Carbon and nitrogen isotope composition of the pre-treatment experiment for archaeological cereal grains of *Triticum aestivum*, *Triticum dicoccum*, * Hordeum vulgare* and *Triticum spelta*. Two concentrations: 1 and 6 M HCl; and two states of grain: entire (e) and powder (p) were tested.
attempted that takes into account the general relationship between $\Delta^{13}C$ and water availability, given that more specific experiments would be necessary in order to better assess the correlation between climatic variables and carbon isotopes in these temperate conditions.

However, the few experiments conducted in well-watered regions or under irrigation have shown that the $\Delta^{13}C$ values from the charred grains of *Triticum aestivum* were higher than 17–17.5‰, indicating a well-watered status (high precipitation/irrigation; Araus et al. 1999; Wallace et al. 2013). Taking this into consideration, it appears that the $\Delta^{13}C$ values of *Triticum aestivum* measured in the archaeological assemblages from the Île-de-France region suggest good prevailing hydric conditions, throughout the studied time period except in two instances. Water availability remained stable throughout the La Tène period, except for the 1st and 3rd century AD, and exhibits lower $\Delta^{13}C$ values. This observation is in agreement with the climate reconstruction of Central Europe by Büntgen et al. (2011), based on the analysis of *Quercus* sp. tree-ring width. The reconstruction of the April to June precipitation indicates two depressions coinciding with our climatic inferences, during years when the June to August temperatures increase. The combination of both variables may have increased the vapour pressure deficit, which can be translated into lower $\Delta^{13}C$ values (Condon, Richards, and Farquhar 1992; Ferrio and Voltas 2005). On the other hand, the well-watered or irrigated barley grains appear in the literature with $\Delta^{13}C$ values of 18–19‰ (Flohr, Jenkins, and Müldner 2011; Wallace et al. 2013). The isotopic values from the charred grains of barley from the both studies regions were higher than from wheat (differences between 0.76 and 1.6‰). The same tendency has been reported in previous studies on archaeobotanical remains and modern material, and has been attributed to distinct growing cycles (Araus, Slafer, and Romagosa 1999; Ferrio et al. 2005; Wallace et al. 2013). Whilst this argument is admitted in dry environments, this explanation may be not be adequate in temperate zones, where distribution of monthly precipitation is very different of Mediterranean climates and the water constraints are less. Nevertheless, these higher values of $\Delta^{13}C$ of barley could provide evidence that wheat was not cultivated in selected areas with better water availability, as the $\Delta^{13}C$ values of wheat are often lower.

In the Champagne region, in spite of a limited number of samples, the lower values of the carbon isotope composition, observed for spelt, most probably reflect low water availability due to the reduced holding capacity of the chalky soils.

**Crop fertility conditions during the La Tène and Roman period**

Considering that the $\delta^{15}N$ values of plants are correlated with the nutrient status of ecosystems (Fogel et al. 2008), and taking into account that the $^{15}N$ signal of cereal grains potentially reflects the overall nutrient quality of agricultural soils, including the effect of manuring (Bol et al. 2005; Bogaard et al. 2007; Fraser et al.
Table 2. Summary of the stable isotopes values, percentage carbon and nitrogen and C:N ratio of Cervus elaphus bone collagen.

| Site            | Bone type | δ¹⁵N (%) | δ¹³C (%) | %N | %C | CN ratio |
|-----------------|-----------|----------|----------|----|----|----------|
| 2-PALAISEAU     | Tibia     | 6.33     | −20.95   | 14.88 | 40.55 | 3.18    |
| Cervus elaphus  | Mandible  | 5.91     | −22.29   | 15.08 | 41.03 | 3.17    |
| n = 10          | Metatarsal| 6.30     | −22.17   | 15.15 | 41.45 | 3.19    |
| Cervus elaphus  | Mandible  | 4.87     | −22.24   | 15.42 | 42.28 | 3.20    |
| n = 10          | Metacarpal| 4.86     | −21.92   | 15.03 | 39.33 | 3.05    |
| Cervus elaphus  | Metatarsal| 5.71     | −21.81   | 12.79 | 35.01 | 3.19    |
| n = 10          | Mandible  | 5.06     | −22.25   | 15.64 | 34.68 | 3.20    |
| Cervus elaphus  | Metatarsal| 6.33     | −21.78   | 15.38 | 41.88 | 3.18    |
| n = 10          | Metacarpal| 4.96     | −21.62   | 15.58 | 37.00 | 3.18    |
| Cervus elaphus  | Metatarsal| 6.53     | −22.12   | 14.19 | 38.96 | 3.18    |
| 9-JOUARS-PONTCHARTRAIN | Calcaneum | 4.50 | −22.14 | 14.99 | 41.47 | 3.23 |
| Cervus elaphus  | Metacarpal| 6.05     | −22.02   | 14.28 | 39.62 | 3.24    |
| n = 10          | Phalange 1| 3.55     | −22.16   | 13.99 | 38.52 | 3.21    |
| Cervus elaphus  | Calcaneum | 3.43     | −22.25   | 15.07 | 41.33 | 3.20    |
| n = 10          | Phalange 1| 4.53     | −22.16   | 15.14 | 41.42 | 3.19    |
| Cervus elaphus  | Talus     | 4.81     | −21.95   | 14.96 | 41.16 | 3.21    |
| n = 10          | Metatarsal| 4.19     | −22.44   | 15.64 | 42.70 | 3.19    |
| Cervus elaphus  | Radius    | 3.10     | −21.49   | 13.66 | 37.67 | 3.22    |
| n = 10          | Metacarpal| 3.95     | −21.84   | 15.67 | 43.07 | 3.21    |
| Cervus elaphus  | Phalange 1| 4.47     | −20.98   | 15.63 | 43.21 | 3.23    |
| n = 10          | Tibia     | 3.83     | −22.14   | 15.05 | 41.14 | 3.19    |
| Cervus elaphus  | Metatarsal| 4.11     | −20.85   | 15.50 | 42.39 | 3.21    |
| n = 10          | Mandible  | 5.22     | −21.76   | 15.12 | 41.66 | 3.21    |
| Cervus elaphus  | Scapula   | 5.79     | −20.85   | 14.70 | 40.72 | 3.23    |
| n = 10          | Metatarsal| 4.59     | −21.87   | 14.61 | 41.12 | 3.28    |
| Cervus elaphus  | Metatarsal| 3.73     | −21.85   | 15.65 | 42.89 | 3.20    |
| n = 10          | Metatarsal| 4.80     | −22.18   | 15.36 | 42.29 | 3.21    |
| Cervus elaphus  | Humerus   | 5.04     | −21.88   | 14.28 | 39.67 | 3.24    |
| n = 10          | Humerus   | 6.58     | −21.74   | 15.68 | 43.22 | 3.22    |
| Cervus elaphus  | Phalange 1| 4.48     | −22.71   | 14.84 | 40.77 | 3.21    |

2011; Szpak et al. 2012), the wide range of δ¹⁵N values measured at these sites implies that cereals were grown in a wide range of soil fertility conditions, and may suggest different manuring rates (Bol et al. 2005; Bogaard et al. 2007). Most assemblages delivered δ¹⁵N values from between 3 and 6‰, reflecting a medium level of fertility; a condition which could result from various scenarios: a light application of manure, the cultivation of new productive lands or the remaining fertility of manured and cultivated lands (Fraser et al. 2011). On the one hand, the cereals from Jouars-Pontchartrain (9) and Palaiseau (2) yielded δ¹⁵N values above 6‰, reflecting a high level or long-term heavy manuring. On the other hand, the cereals from Bonneuil (5) and Acy-Romance (11) yielded low δ¹⁵N values, which could reflect impoverished or long-term unmanured soils.

At Jouars-Ponchartrain (9), similarly high δ¹⁵N values were measured in wheat and barley, which suggests that the same cultural practices were applied to both species. Conversely, interspecific differences appeared in all other sites, implying heterogeneous strategies of cultivation, depending on the cereal species (Aguilera et al. 2008).

In general, Triticum aestivum (naked wheat) presents higher δ¹⁵N values than hulled wheat (Triticum dicoccum or Triticum spelta) when cultivated contemporaneously at the same site; exceptions to this are the earliest assemblages of Bailly (1; 5th century BC) and Morigny-Champigny (10; 3rd century BC). During La Tène I, naked wheat was probably grown in marginal fields; then, later on, the preference for naked wheat increased and it started to be cultivated as the main crop in selected areas or on manured soils (Zech-Matterne, Wiethold, and Pradat 2014).

In the Champagne region, the δ¹⁵N values of spelt were higher than those measured in barley.

While Näsholm, Huss-Danell, and Hogberg (2000) demonstrated that could exist interspecific differences in δ¹⁵N values of agricultural grasslands caused by various N-uptake patterns and N assimilation. Fraser et al. (2011) demonstrated similar effect of manuring on cereal grain δ¹⁵N in different species. Therefore, the observed difference between crop species could be explained either by distinct cropping systems, where the best soils would be allocated to wheat species; or different manuring levels applied to different species; or a difference in the cereal ability to recover ¹⁵N from organic manure depending of interspecific competition with weeds (Ruisi et al. 2015).

Manuring practices in the cropping systems

There are multiple conditions that can cause higher δ¹⁵N values; one of which is scarce precipitation: a

Figure 4. δ¹⁵N and Δ¹³C of domestic cereals, bone collagen of Cervus elaphus and estimated Cervus elaphus diet.
negative correlation that has been reported between mean annual precipitation and leaf $\delta^{15}$N (Handley et al. 1999; Amundson et al. 2003), even though Fraser et al. (2011) detected a positive relationship between the $\delta^{15}$N values of cereals grown in manure fields and mean annual precipitation, the same study no correlation between $\delta^{15}$N values of cereals cultivated in unmanaged fields and precipitation was evidenced. Another cause of higher $\delta^{15}$N values is high temperature, given that cold and humid systems tend to preserve and recycle N (Handley et al. 1999). However, climatic conditions were not responsible for the increase in $\delta^{15}$N values here; since the general trend inferred by the $\Delta^{13}$C values indicate good growing environmental conditions for wheat and barley, in agreement with reconstructed precipitation and temperature from oaks (Büntgen et al. 2011). Another influential factor which could have caused the high $\delta^{15}$N values is salinity, but this was ruled out as the current flora composition of the region, and the weeds recovered from the archaeological sites, did not show a significant frequency of salt tolerant plants (Zech-Matterne and Brun 2016).

The high $\delta^{15}$N values of domestic plants could indicate naturally rich soils or farming practices. The $\delta^{15}$N values from red deer bone collagen can be used to estimate the fertility of unmanaged soils and distinguish between natural causes and intentional management. At Palaiseau (2) and Jouars-Pontchartrain (9), which delivered among the highest $\delta^{15}$N values in the Île-de-France, soil fertility was probably enhanced through manuring, leading to a significant rise in the $\delta^{15}$N values of cereals in comparison to those estimated for the wild vegetation (Figure 4). On the contrary, at Acy-Romance (11) in Champagne, where the $\delta^{15}$N values measured in red deer bone collagen were very similar to those obtained at Jouars-Ponchartrain, suggesting a similar $\delta^{15}$N baseline value for non-fertilised plants; the $\delta^{15}$N values measured in cereal crops were similar to those estimated for plants grown on unmanaged soils, suggesting no fertilisation practices in this agrarian system of the Champagne region.

The highest difference between cultivated cereals and wild plant $\delta^{15}$N values was reported at Jouars-Pontchartrain (ca. +7.0 ‰). This considerable $^{15}$N enrichment in cereals may be due to a distinct quality of animal manure. While the effect of animal manure on plants $\delta^{15}$N may vary greatly, due to numerous variables constraining N-uptake and assimilation by plants (Szpak 2014), the origin of animal fertiliser can also influence $^{15}$N-enrichment: poultry or cattle produce faeces with slightly lower $\delta^{15}$N values than caprines and considerably lower than pigs. In keeping with most archaeological sites from these periods, the relative proportion of animal species within the assemblages was essentially composed of five domesticates: cattle, pig, sheep, horse and dogs; the latter two being consumed during the La Tène period. However, different proportions were noted across sites and within sites, depending on the areas excavated, the different nature of the archaeological structures and the living standards of the inhabitants. It is therefore difficult to define a unique snapshot of food and breeding at a specific site: some areas may have delivered numerous cattle or horse bones, while other places may have been rich in sheep or pig remains. Overall, the feeding at Acy-Romance was based primarily on beef and horse. At Jouars-Pontchartrain sheep and cattle were well represented, as were pigs from several domestic rubbish pits; the faeces from all of these animals could have been potentially used to manure the fields. Human waste or sewage could also have been used at this site, as documented in the written records of the Roman period (Bakels 1997; Cordier 2003; Poirier and Nuninger 2012).

Palaiseau presents a consistent trend in cereal $\delta^{15}$N values along the analysed temporal sequence from 2nd century BC to 2nd century AD. This consistency, during 300 years of cultivation, is also in favour of human management; and implies a good knowledge of fertilisation practices for the purpose of improving and sustaining the fertility of cultivated soil.

Conclusions

This multiscale stable isotope investigation on crop fertility conditions revealed interesting aspects of interregional, intersite and interspecies variability. This was due to the abundance of carpological assemblages from the Iron Age to the Roman period, noticeably more consistent than from most Neolithic contexts, and strong preliminary knowledge on crop cultivation systems. Interregional differences in edaphic conditions, of the lower water holding capacity of the Champagne chalky soils compared to the Île-de-France luvisols, was reflected in lower $\Delta^{13}$C values for spelt cultivated in the former region. In Acy-Romance and Champfleury in Champagne, no manuring was applied to correct the lower fertility of soils, but was managed by the selection of crop species (spelt and barley) better suited to the prevailing soil conditions.

Alternatively, in the Île-de-France region, the $\delta^{15}$N values measured in charred grains showed high intersite variability, suggesting a wide range of soil fertility conditions, most probably linked to different manuring rates and history. Where an intrasite diachronic approach was rendered possible, different time trajectories were also highlighted between sites; suggesting, again, a significant influence of cultivation practices on similar soil substrates. Within each site, intercrop variability was more difficult to interpret, given that it may partly include internal differences in crop physiology or different cropping systems. Hopefully further
studies in this area, preferentially on ancient varieties of cereals, will successfully address this issue. It was noted that intercrop variability was not systematic, suggesting that it may reflect different treatments for different cereal types. In most cases, higher $\delta^{15}N$ values were measured in naked wheat compared to hulled wheat from the same site, which may be explained by the particular care given to naked wheat.

The use of animal manure was clearly demonstrated at Palaiseau and Jouars-Ponchartrain. At Jouars-Ponchartrain, the 7% $^{15}N$-enrichment in cultivated cereals, compared to the estimated values for unmanured plants, highlighted the need to identity the actual fertiliser used (i.e. cattle or caprines/pig or human). A closer examination of the association between plant and animal domesticates (Lepetz and Matterne 2003) may help to clarify this. The relationship between crop and animal husbandry may also include a return from the manured plant to the animal in the form of fodder. This particular topic is the subject of ongoing work in this region.

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