Nitrogen and Carbon Stable Isotope Analysis of Holocene Pigs from Liang Bua (Flores, Indonesia)

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Abstract

In this study, pigs from Liang Bua (Flores, Indonesia) are examined using nitrogen and carbon stable isotopes to explore possible changes to their diet over the past ~5,000 years. This temporal period is of particular interest because major changes occurred at Liang Bua during this time and these changes are thought to be related to the dispersal of Austronesian people and/or culture. Most importantly, this research attempts to address whether pig diet changed in relation to the onset of farming activities in the area surrounding Liang Bua ~3 ka and, if so, in what ways. Stable isotope values (δ^{13}C and δ^{15}N) were obtained for 57 (39 bone, 18 tooth) pig specimens from Liang Bua that spanned in age from ~4,300 until ~400 years ago, and one horse and seven human samples from the site were used to provide a comparative context. Results of this study show that the δ^{13}C values of pigs and humans changed at Liang Bua after the onset of farming ~3,000 years ago, as did the δ^{15}N values of pigs. Changes in δ^{13}C values suggest that beginning after ~3,000 years ago, one or more types of C_4 plants were consumed by pigs and humans, leading to more enriched δ^{13}C values compared with earlier populations. Foxtail millet, a C_4 plant long associated with the dispersal of Austronesian people throughout island Southeast Asia, is the most likely candidate for a C_4 plant used as an agricultural crop around Liang Bua during that time. Changes in the δ^{15}N values of pigs suggest that after ~3,000 years ago, the diet of some of the Liang Bua pigs was restricted, resulting in lower δ^{15}N values than observed among any pigs from earlier time intervals. Furthermore, these ‘captive’ pigs did not have access to the newly available C_4 resources. The custom of keeping domesticated pigs in small enclosures and provisioning them with mostly C_3 plants, practiced by Indigenous Manggarai people living around Liang Bua today, is the most likely explanation of these results.

Introduction

Liang Bua is a limestone cave that is located on the Indonesian island of Flores, which is situated directly in between the Asian and Australian continents (Brown et al., 2004; Morwood et al., 2004). Although best known as the type site of *Homo floresiensis*, Liang Bua also preserves a relatively complete Holocene stratigraphic sequence that is deeply stratified with dense accumulations of faunal remains, stone artifacts, and pottery (Moore et al., 2009; Morwood et al., 2009; Sutikna et al., 2018, 2020; Veatch et al., 2019, 2020; Julianto et al., 2020; Lin et al., 2020). Of these Holocene faunal remains, pigs are the most abundant of the large mammals (i.e., body mass ≥ ~5 kg) (Sutikna et al., 2018). Pigs are not endemic to Flores as they are absent from all known Pleistocene sites on the island (van den Bergh et al., 2009; Sutikna et al., 2018, 2020). Their first recorded appearance on the island occurs at Liang Bua in the early Holocene ~7,000 years ago (van den Bergh et al., 2009), during which time they were quite rare (Sutikna et al., 2020). However, they become noticeably abundant relative to other vertebrates in the faunal assemblage beginning ~3,600 years ago (Sutikna et al., 2020), during a ~1,000-year period when the harvesting of freshwater shellfish appears to have been a main focus of modern human (*Homo sapiens*) populations in the area (Julianto et al., 2020). Previous work has suggested that the earliest pigs at Liang Bua were probably *Sus celebensis* (the Sulawesi warty pig) while the later more abundant pig remains were likely *Sus scrofa* (the Eurasian pig) (Larson et al., 2007; van den Bergh et al., 2009), but further morphological and genetic analyses are needed for confirmation. It is certainly possible that both pig species are represented at Liang Bua throughout most of the Holocene and that some of these pigs were domesticated.

Several other mammals not endemic to Flores initially appear at Liang Bua between ~3,600 to 3,000 years ago, including macaques, porcupines, and civets (Evans et al., 2020a,b;

Sutikna et al., 2020). Pottery first appears ~3,000 years ago at the site suggesting the onset of farming activities in the area (Sutikna et al., 2018; Julianto et al., 2020). Indeed, a noticeable shift to including C_4 plants in the diet is observed in modern humans, pigs, and other animals at Liang Bua beginning ~2,700 years ago (Anderson, 2011; Munizzi, 2013; Tocheri et al., 2020). This dietary shift in modern humans is also associated with a component of Austronesian genetic ancestry according to preliminary analysis of ancient DNA from skeletal remains directly dated to ~2,700 and ~2,600 calibrated radiocarbon years before present (cal. years BP) and buried with Austronesian-style grave goods, including pottery and a polished adze (Julianto et al., 2020; Oliveira et al., 2020; Tocheri et al., 2020).

In this study, nitrogen and carbon stable isotopes are used to explore possible further dietary and/or environmental changes over the past ~5,000 years for pigs as well as modern humans at Liang Bua. As mentioned above, pigs are not found in the Liang Bua stratigraphy prior to the Holocene (van den Bergh et al., 2009; Sutikna et al., 2018, 2020). Instead, the earliest pig fragments identified thus far at the site are ~7,000 years old (van den Bergh et al., 2009) but otherwise are relatively scarce until after ~3,600 years ago (Sutikna et al., 2020). The sudden increase in abundance ~3,600 years ago suggests that pigs were intentionally brought to Flores by modern humans, either through trade or population dispersal (Larson et al., 2007; Morwood et al., 2009; van den Bergh et al., 2009; Sutikna et al., 2018, 2020). On other Indonesian islands (e.g., Sulawesi's Maros karst region), multiple prehistoric rock art paintings document the longstanding importance of pigs to humans (Aubert et al., 2014; Brumm et al., 2021). For instance, an image of a pig identified as *Sus celebensis* was painted on the walls of Leang Tedongnge at least 45,500 years ago (Brumm et al., 2021) while at Leang Timpuseng another image of a pig identified as babirusa (*Babyrousa* sp.), which means 'pig-deer' in Indonesian, is at least 35,400 years old (Aubert et al., 2014). Pig remains have also been recovered from archaeological sites across Indonesia and appear to have played important roles (e.g., dietary, social, ceremonial, etc.) for human populations throughout the past several thousand years (van den Bergh et al., 2009; Fajari and Kusmartono, 2013; Suroto, 2014; Bellwood, 2017; Sutikna et al., 2018, 2020; Ono et al., 2020; Shipton et al., 2020).

Stable isotope analyses

Isotopes are defined as atoms with the same number of protons (i.e., the same element) but different numbers of neutrons (Sharp, 2017). Atoms consist of specific combinations of protons, electrons, and neutrons. A carbon atom, for example, always has six protons and six electrons but the exact number of neutrons varies among the isotopes of this element. Roughly 98.9% of all carbon atoms have six neutrons. As the atomic mass of an atom equals the summed total number of protons and neutrons, most carbon has an atomic mass of 12, which is notated as ^{12}C. However, about 1.1% of all carbon atoms have seven neutrons, resulting in an atomic mass of 13, notated as ^{13}C. Both ^{12}C and ^{13}C are stable isotopes because they do not decay into other elements (Sharp, 2017). In contrast, unstable (or radioactive) isotopes do decay into other elements due to an imbalance in their proton and neutron compositions. Approximately one out of every trillion ^{12}C atoms has eight neutrons, resulting in an atomic mass of 14, or ^{14}C. This particular isotope of carbon is unstable as it decays into nitrogen (^{14}N). Because the rate of decay is consistent over time (half-life of ~5,730 years), ^{14}C is useful for estimating the number of years since a plant or animal died and stopped ingesting new carbon (Cherry, 2009; Kutschera, 2019).

Stable isotopes are measured using the ratio of heavier and lighter isotopes compared to the ratio of a reference standard material such as Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen (Ambrose, 1993; Sharp, 2017). Isotope values are stated by using delta (δ) notation in parts per thousand, or per mil (‰) (Ambrose, 1993; Sharp, 2017). When the ratio of heavy to light isotopes is higher in the sample than it is in the standard that anchors the reference scale (e.g., VPDB or AIR), the results of the δ value would be positive, whereas negative values of δ mean that the ratio of heavy to light isotope is lower in the sample than it is in the standard (Sharp, 2017). Coplen (2011) provides the following equation for expressing the δ value,

$$\delta = (R_{sample} / R_{standard}) - 1,$$

where R_{sample} represents the isotopic ratio of the sample and $R_{standard}$ represents the isotopic ratio of the standard.

Isotope analysis can be conducted using any material that contains the element of interest, including (but not limited to) breath CO_2, blood and other fluids, hair, skin, fingernails, muscle, fat and other soft tissues, urine and feces, and bone or tooth carbonate and collagen (Ambrose, 1993). Bone stable isotope analysis and ^{14}C dating are typically accomplished on extracted collagen. Thus, collagen preservation is critically important because the quality of bone collagen extract is integral to any subsequent isotope analysis (van Klinken, 1999). The preservation of bone collagen can vary considerably between and within archaeological sites. A suite of factors, including localized burning, cooking, variation in sedimentary matrix chemistry (i.e., acidity, oxygenation, and bacterial action), age, duration of exposure prior to burial, and bone and tooth density, can all affect the degree to which collagen is preserved (Gordon and Buikstra, 1981; Ambrose, 1990; López-Costas et al., 2016). Collagen preservation is also affected by temperature and/or moisture levels. For example, higher temperatures typically result in higher levels of collagen degradation in comparison with lower temperatures, and this partially explains why collagen is usually better preserved in temperate zones than it is in the tropics (van Klinken, 1999). Similarly, collagen is usually poorly preserved in arid environments compared with humid environments, which typically have well-preserved collagen (van Klinken, 1999). Bone collagen loss often occurs more rapidly in tropical than temperate environments, but what collagen remains tends to be intact (i.e., has not undergone selective amino acid loss) and suitable for isotopic analysis (Pestle and Colvard, 2012).

Stable isotope measurements are used to answer research questions in various fields including geochemistry, climatology, hydrology, botany, physiology, ecology, archaeology, meteorology, meteoritics, paleobiology, bacteriology, and paleontology (Sharp, 2017). In archaeology, stable isotope analysis typically contributes to reconstructions of past environments as well as the past diet and mobility of humans and other animals. Such reconstructions are typically obtained by measuring the isotopic values of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$), hydrogen ($^{2}H/^{1}H$ or D/H) and strontium ($^{87}Sr/^{86}Sr$), although other isotopes are sometimes used as well (Britton et al., 2011; Metcalfe et al., 2011; Pryor et al., 2013; Stevens et al., 2014; Szostek et al., 2015; Sharp, 2017; Fenner et al., 2018).

Nitrogen stable isotopes

Nitrogen stable isotope values ($\delta^{15}N$) are typically used to investigate the diet and trophic level (i.e., the position an organism occupies within the food web of its ecosystem) of animals in terrestrial and marine food webs (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). At the base of every food web are primary producers (e.g., plants) and their $\delta^{15}N$ values and variability are critically important to understand as these values act as the starting point from which all subsequent consumers' $\delta^{15}N$ values are derived (DeNiro and Epstein, 1981; Szpak et al., 2013; Szpak, 2014). There are a number of different factors that influence the $\delta^{15}N$ values of plants in any given ecosystem. Some plants obtain nitrogen directly from the atmosphere, in which case their δ^{15}N values are close to 0‰, the same value as N_2 in the atmosphere (Szpak et al., 2013). Meanwhile, other plants absorb their nitrogen entirely from the soil. In this case, the nitrogen derives from compounds such as ammonium (NH_4^+) or nitrate (NO_3^-), which are broken down from decomposed organic matter (Katzenberg, 2008; Szpak, 2014).

Many other factors (e.g., taxonomy, physiology, environment) can result in considerable natural variation in plant nitrogen isotopic composition (Szpak et al., 2013). For example, aquatic plants often have δ^{15}N values that are higher on average than in terrestrial plants (Cloern et al., 2002), graminoids and forbs tend to have high δ^{15}N values relative to those of shrubs and trees (Bocherens, 2015), and nitrogen-fixing plants, such as legumes, typically have lower δ^{15}N values compared to other terrestrial plants (Szpak, 2014). There may also be within-plant and/or temporal variation, such as different assimilation processes in the root versus the shoot parts of the plant, different nitrogen movement between sources and sinks, and reliance on isotopically variable sources of nitrogen as plant tissue forms over time (Szpak et al., 2013). In agriculture, using fertilizer is often required to give the necessary nutrients to the soil and this can also result in an increase in plant δ^{15}N values (Choi et al., 2002; Szpak, 2014). For example, the utilization of solid waste and slurry manures can increase the δ^{15}N values of plants up to +20‰, depending on the δ^{15}N value of the manure (Choi et al., 2002; Yun et al., 2006; Bateman and Kelly, 2007; Szpak, 2014). The use of seabird guano as fertilizer can also increase plant δ^{15}N values between +10‰ and +40‰ (Szpak, 2014). Other factors, like anthropogenic burning or shifting cultivation can increase plant δ^{15}N values between about +2‰ and +8‰, depending on the original vegetation (Huber et al., 2013; Szpak, 2014).

Environmental factors create conditions that may vary from hot and dry to cold and wet and these conditions impact not only the δ^{15}N values of plants but also the transfer of nitrogen through the entire food web. Colder and wetter conditions result in food webs that conserve and recycle nitrogen, resulting in lower δ^{15}N values in comparison to those with hotter and drier conditions, which promote larger isotopic fractionations associated with nitrogen loss (Schwarcz et al., 1999; Amundson et al., 2003; Szpak et al., 2013). Aridity and/or the general availability of water strongly impacts the δ^{15}N values of plants and animals (Ambrose, 1993; Amundson, 2003; Schwarcz et al., 1999) and it is typical for plants that grow in more arid localities to have relatively high δ^{15}N values (e.g., Metcalfe and Mead, 2019). In some cases, the δ^{15}N values of terrestrial animals living in arid environments may be higher than those of marine animals (Ambrose, 1993). In the Dakhleh Oasis, Egypt, where precipitation is close to zero, wild and cultivated plants, domestic animals, and humans all had relatively high δ^{15}N values compared to their counterparts in other regions with higher precipitation (Heaton et al., 1986; Heaton, 1987; Schwarcz et al., 1999). A similar relationship was also found in a comparison of kangaroo bones from arid areas with low precipitation (δ^{15}N values of +8.8‰ to +12.6‰) with those from temperate areas with high precipitation (δ^{15}N values of +3.5‰ to +6.8‰) in Southern Australia (Pate and Anson, 2018). Therefore, environmental factors and especially precipitation must be considered when evaluating δ^{15}N values in both plants and animals because they may significantly impact either the base δ^{15}N values within the ecosystem and/or the changes in δ^{15}N values from one trophic level to the next.

For each successive trophic level, δ^{15}N values typically increase. The first careful study of this relationship between δ^{15}N values in food and its consumer found that δ^{15}N increased between +1.3‰ and +5.3‰ from one trophic level to the next, with an average of +3.4‰

(Minagawa and Wada, 1984). For mammals, a trophic level increase of +4.0 ± 0.74‰ in δ^{15}N is now generally accepted for both primary producer (e.g., plant) to primary consumer (e.g., herbivore) and primary to secondary consumer (e.g., carnivore) (O'Connell et al., 2012; Cheung and Szpak, 2020). However, the exact magnitude of this trophic shift is extremely variable and depends on numerous factors, including diet (McCutchan et al., 2003; Sponheimer et al., 2003; Robbins et al., 2005; Hedges and Reynard, 2007; Caut et al., 2009; Warinner and Tuross, 2010; O'Connell et al., 2012; Leichliter et al., 2021).

Some other factors that can impact the δ^{15}N value in mammals include nutritional stress, depressed growth, and breastfeeding/nursing. For example, nutritional stress resulted in the δ^{15}N values of wild bison (*Bison bison athabascae*) to increase from between +4.0‰ and +6.5‰ to between +7.4‰ and +9.4‰ (Funck et al., 2020). Another study found that nutritional stress caused by morning sickness during early pregnancy resulted in the δ^{15}N values of eight women to increase between +0.3‰ and +1.0‰ from their values measured pre-pregnancy (Fuller et al., 2005). However, when their pregnancies were nearing full-term, their δ^{15}N values decreased by 0.4‰ to 1.7‰ because of weight gain and lack of nutritional stress (Fuller et al., 2005). A similar result was observed in a controlled diet experiment, where a juvenile pig that experienced depressed growth (i.e., less weight gain) relative to other juvenile pigs showed elevated δ^{15}N values +0.9‰ higher in its hair keratin and +1.1‰ higher in its bone collagen (Warinner and Tuross, 2010). This result occurred even though all of these pigs were kept in the same place, had access to the same foods, and were allowed to eat, drink, and exercise freely (Warinner and Tuross, 2010). Finally, nursing is another factor that can result in enriched δ^{15}N values in juvenile mammals. In humans, the hair and fingernails of infants that are exclusively breastfed typically have δ^{15}N values that are enriched by about +2‰ to +3‰ compared to their mother

(Fuller et al., 2006). In mammalian bone collagen, however, the δ^{15}N values of nursing juveniles is also expected to be higher than in the mother but probably significantly less than a full trophic level and highly variable across species (Reynard and Tuross, 2015). For these reasons, δ^{15}N values in primary and secondary consumers may vary considerably, depending on what kind or combination of foods they eat, the specific processes that are affecting the consumed foods, and the various factors influencing the amount of trophic level increase.

Carbon stable isotopes

Carbon stable isotope values (δ^{13}C) are typically investigated in combination with δ^{15}N and sometimes other stable isotopes to reconstruct the paleodiet of animals (Bocherens, 2015). In terrestrial ecosystems, plants vary in the metabolic pathways (C_3, C_4, or Crassulacean Acid Metabolism [CAM]) they use during photosynthesis (Hatch and Slack, 1967). During the first stage of photosynthesis, C_3 plants use three-carbon compounds whereas C_4 plants use four-carbon compounds (Hatch and Slack, 1967). C_3 plants are the most prevalent plants on earth with ~95% of species using this photosynthetic pathway (Sage, 2016; Boretti and Florentine, 2019). In contrast, ~5% of plant species use the C_4 photosynthetic pathway and a small number of additional plant species use the CAM photosynthetic pathway (Sage, 2016; Boretti and Florentine, 2019). C_3 plants that are often consumed by humans and/or other animals include wheat and rice, forest and wetland grasses, all root crops, legumes, vegetables, nuts, and most fruits; common dietary C_4 plants include sorghum, millets, maize, sugarcane, some amaranths, chenopods, and tropical pasture grasses; and CAM plants include cacti, euphorbias, agaves, and bromeliads (pineapples) (Ambrose, 1993).

Each of the three photosynthetic pathways has different temperature and humidity requirements. C_3 plants grow better in conditions that are cool and wet with optimum temperatures ranging between 18° and 24°C whereas C_4 plants grow better in hot and dry conditions with optimum temperatures ranging between 32° and 55°C (Boretti and Florentine, 2019). C_4 plants mostly occur in relatively arid regions where there is a combination of high temperatures and some water stress (Sage, 2004, 2016) whereas CAM plants are adapted for desert climates where water is extremely scarce and temperatures between night and day shift considerably (Yamori et al., 2014). The $\delta^{13}C$ values of C_3, C_4, and CAM plants vary greatly, with modern C_3 plants ranging from about –35‰ to –20‰, C_4 plants from about –14‰ to –9‰, and CAM plants from about –20‰ to –10‰ (O'Leary, 1988; Katzenberg, 2008). Since the $\delta^{13}C$ values of C_3 and C_4 plants do not overlap with each other, and because these are passed on up the food chain, it is possible to identify pure C_3 or pure C_4 consumers and to estimate the proportion of C_3 to C_4 plants in a mixed-feeder's diet (Deines, 1980; Katzenberg, 2008; Cheung and Szpak, 2020). However, one first must account for the small trophic increase in $\delta^{13}C$ that occurs from food to consumer, as well as isotopic differences among tissues, which are mostly due to the different amino acid compositions of bone collagen and plants (DeNiro and Epstein, 1978; Deines, 1980; van der Merwe, 1982; Katzenberg, 2008; Cheung and Szpak, 2020).

Bone collagen in herbivorous mammals is generally enriched in $\delta^{13}C$ by +5.0 ± 0.63‰ compared with dietary plants (Cheung and Szpak, 2020). In contrast, the bone collagen $\delta^{13}C$ values of omnivores/carnivores are typically about +1.0 ± 0.63‰ higher than the bone collagen $\delta^{13}C$ values of the animals they consumed, and this is known as the trophic enrichment factor (Cheung and Szpak, 2020). In previous research using pigs fed a mixed C_3 and C_4-based vegetarian diet, the $\delta^{13}C$ value of pig bone collagen was +4.2‰ higher than that of the plants they consumed (Warinner and Tuross, 2009). Therefore, pigs that are pure C_3 consumers would be expected to have bone collagen $\delta^{13}C$ values that range between about –31‰ and –16‰, whereas pigs that are pure C_4 consumers could have $\delta^{13}C$ values that range between about –10‰ and –5‰. These ranges could also be slightly more enriched (up to about +1.0‰) depending on any trophic effect from eating animal-based protein but overall, they are generous estimates for pure C_3 or C_4 consumers as the actual ranges of values within any given population of consumers would probably be smaller. It is unlikely that a pure C_3 or C_4 consumer would have values at the most extreme ends of these ranges because they would have to eat only foods with the highest or lowest values in each range. Moreover, such foods are not necessarily available everywhere and even when they are available, herbivores are unlikely to feed exclusively on those foods.

Isotopic studies of pig domestication

Stable isotope values offer information about animal diets in particular temporal, spatial, and environmental contexts, and several previous stable isotope studies either focused on pigs or included them within a broader sample (e.g., Minagawa et al., 2005; Hu et al., 2008; Rosvold et al., 2010; Halley and Rosvold, 2014). Changes in stable isotope values, $\delta^{15}N$ in particular, have been used as proxies for pig domestication in previous studies (Halley and Rosvold et al., 2014; Hu et al., 2008; Minagawa et al., 2005; Pechenkina et al., 2005). These studies interpreted a larger range of pig $\delta^{15}N$ values at sites in Norway, Japan, and China as evidence for either human control of pig diets or pigs having access to human leftover food, both of which were presented as evidence that some of the pigs were domesticated. Using stable isotope data in this way rests on the assumption that the diets of domesticated and wild pigs will differ due to human control of the foods available to domesticated pigs, either through direct feeding or from restricting the area in which the pig can forage (Rowley-Conwy et al., 2012). However, the diet of both domesticated and wild pigs is far more complex than these previous studies suggest. Pigs are highly opportunistic omnivores (Masseti, 2007; Rowley-Conwy et al., 2012) so it may not be possible to predict beforehand the direction of changes in stable isotope values associated with domestication. Wild and domestic pigs can potentially be differentiated using metric and non-metric methods, including the length of the upper and lower third molars, linear enamel hypoplasias, and microwear analysis (Rowley-Conwy et al., 2012). Compared to domesticated pigs, wild pigs typically have larger third molars, lower levels of linear enamel hypoplasias, and higher amounts of dental microwear (Ward and Mainland, 1999; Dobney et al., 2004; Magnell and Charter, 2007; Rowley-Conwy et al., 2012). Therefore, in ideal settings, data from multiple sources in addition to isotopes (e.g., body mass, DNA, morphological and taphonomic details, stable isotopes, etc.) should be used to test whether domestication has occurred (Dobney et al., 2004; Larson et al., 2007; Magnell and Charter, 2007; Ottoni et al., 2012; McHugo et al., 2019).

Some studies (reviewed below) specifically attempted to discern between wild and domesticated pigs using δ^{13}C and δ^{15}N only. Minagawa et al. (2005) investigated δ^{13}C and δ^{15}N in pigs from island sites of Japan between 1,700 and 7,000 years old. Their results showed that pigs from the islands of Honshu and Kyushu had almost the same average δ^{13}C values as pigs from Ryukyu Island (–21.0 ± 1.3‰ and –20.0 ± 1.7‰, respectively), both of which indicate a C_3-based diet (Minagawa et al., 2005). The average δ^{15}N value of pigs from Ryukyu Island (+9.2 ± 1.8‰) was significantly higher than in pigs from Honshu and Kyushu Islands (+4.1 ± 1.1‰), although the overall range of δ^{15}N values for pigs from Ryukyu overlapped with that seen on the other islands (Minagawa et al., 2005). In comparison, deer from Honshu and Kyushu had δ^{13}C values of –21.5 ± 1.4‰ and δ^{15}N values of +4.2 ± 1.0‰, and these were not significantly different than those of the pigs from these particular islands (Minagawa et al., 2005). Minagawa et al. (2005) argued that some of the pigs on Ryukyu Island were domesticated because their δ^{15}N values were enriched relative to those of deer. They suggested that these pigs had access to leftover food from humans, whereas wild pigs had lower δ^{15}N values due to limited access to such resources (Minagawa et al., 2005). While it is certainly possible that these particular pigs had access to leftover food from humans (or perhaps other human waste), pigs are highly opportunistic animals in terms of diet. For example, Minagawa et al. (2005) themselves mentioned that wild pigs from the Ishigaki islands eat sugarcane (a domesticated C_4 plant), demonstrating that wild pigs can sometimes access domesticated crops without being domesticated themselves. Although the dietary habits of deer on Honshu, Kyushu, and Hokkaido islands do not appear to vary over the past 6,000 years (Minagawa et al., 2005), deer are natural browsers that may often graze whereas pigs are opportunistic omnivores. Consequently, wild pigs that scavenged human refuse would likely have higher δ^{15}N values than pigs that did not and therefore distinguishing between wild and domesticated pigs requires additional empirical and/or contextual evidence.

Hu et al. (2008) investigated shifts in δ^{13}C and δ^{15}N values of four pigs from Yuezhuang, China that date to about 8,500–7,500 years ago. Two of these pigs had δ^{13}C values of –18.1‰ and –20.0‰ along with δ^{15}N values of +4.7‰ and +6.0‰, respectively, and both were interpreted as wild pigs because their δ^{13}C values reflect a C_3 plant-dominated diet (Hu et al., 2008). A third pig had a δ^{13}C value of –10.6‰ and a δ^{15}N value of +6.4‰ and was interpreted as domesticated because its δ^{13}C value reflects a mostly C_4 plant diet, possibly due to the inclusion of millet stems and hulls (Hu et al., 2008). However, as discussed above, wild pigs can often access domesticated crops. Hu et al. (2008) also considered the fourth pig as domesticated because its δ^{15}N value of +9.1‰ was relatively close to those of humans in their sample even though its δ^{13}C value of –19‰ indicated a typical C_3 plant diet that was different than observed in their human sample. It was argued that the high δ^{15}N value of this pig was the result of it consuming leftover food from humans (Hu et al., 2008). As mentioned above, while these varying isotopic values for these four pigs certainly indicate dietary differences among them, they do not necessarily indicate that two of them were domesticated.

Another study examined the δ^{15}N and δ^{13}C values of 21 pigs from the Norwegian site of Skipshelleren (Rosvold et al., 2010). Pigs from the pre-agricultural period had a mean δ^{15}N value of +3.6‰ whereas the mean δ^{15}N value for pigs from the agricultural period was +4.8‰ (Rosvold et al., 2010). The δ^{13}C values for these two groups of pigs were relatively constant with –22.0‰ before and –21.6‰ after agriculture was adopted, likely because the domesticated crops used during the agricultural period were C_3 rather than C_4 plants (Rosvold et al., 2010). Rosvold et al. (2010) suggested that the increased δ^{15}N values observed in the pigs from the agricultural period was likely due to higher amounts of animal protein in their diet possibly because these pigs were fed human food refuse such as leftover fish or meat (Rosvold et al., 2010). Moreover, they suggested that this slight increase in δ^{15}N value was evidence that these pigs were domesticated (Rosvold et al., 2010). Although the δ^{15}N of pigs during the period in which the transition to farming was occurring were more varied than at any other temporal interval and there was a slight (but not statistically significant) increase in the δ^{15}N of pigs after this transition (Rosvold et al., 2010), it is not necessarily evidence that the later group of pigs were domesticated. Certainly, pig domestication is one possible explanation for this observed change in diet, but wild pigs could also have been accessing new dietary resources made available due to human behaviours (e.g., burning or manuring related to agricultural activities) (Szpak, 2014) or scavenging human waste or garbage. More problematic, however, is that this previous study did not present or consider any quality control indicators, such as the C:N ratio of the examined stable isotope values. The C:N ratio is critically important to assess the collagen preservation of the samples and without this information it should not be assumed that the isotopic values accurately reflect the diets of these animals (Schoeninger et al., 1989; Wright and Schwarcz, 1999).

In a subsequent study, Halley and Rosvold (2014) compared the nitrogen and carbon stable isotopes of 22 pigs from Skipshelleren with those of seven pigs from the site of Dreggsallmenningen, a Medieval site in Bergen, Norway. The samples and stable isotope values of pigs from Skipshelleren were similar but not identical to the previous study (Rosvold et al., 2010) possibly because the more recent study (Halley and Rosvold, 2014) excluded some samples that had C:N ratios outside the acceptable range of 2.9–3.6. Pigs from Medieval Dreggsallmenningen had average δ^{15}N and δ^{13}C values of +10.4‰ and –20.7‰, respectively, whereas pigs from Skipshelleren (which included specimens from pre-agricultural, agricultural transition, and agricultural periods) had average δ^{15}N and δ^{13}C values of +3.9‰ and –21.9‰, respectively (Halley and Rosvold, 2014). Halley and Rosvold (2014) suggest that the Medieval pigs had more C_4 plants and protein (probably marine fish) in their diets and almost certainly received most of their food directly from humans. However, it is not necessarily more protein in the pigs' diet, but rather, foods with higher δ^{15}N values, which could be marine or highly fertilized plants, for example. In contrast, the Skipshelleren pigs from pre- and post-agricultural periods have similar ranges of δ^{15}N and δ^{13}C values in comparison to one another and were not tested statistically (Halley and Rosvold, 2014) suggesting that there may have been no significant difference between the pigs' diet in both periods.

Finally, Guiry et al. (2017) found that the average of δ^{15}N of 65 pigs from rural sites in Upper Canada was +6.9 ± 1.6‰, while the average of 59 pigs from urban sites in upper Canada was also +6.9 ± 1.6‰. However, the average δ^{13}C value at rural sites was –21.2 ± 0.9‰, which suggests a pure C_3-based diet, whereas urban sites have an average $\delta^{13}C$ value of –20.2 ± 1.1‰, but with a wider range (–22.9‰ to –11.4‰) indicating a variety of C_3 and C_4 plant consumption (Guiry et al., 2017). Guiry et al. (2017) suspected that there were social and economic differences among urban sites, such as for the wealthier urban site, where people may have consumed pigs that were fed with maize and/or were imported from the USA. In contrast, for less wealthy urban sites, people likely consumed locally raised pigs that were only fed with C_3 plants (Guiry et al., 2017).

All of the previous studies indicate that variations in the $\delta^{15}N$ and $\delta^{13}C$ values of pigs primarily depend on pig diet, but that pig diets can be mediated by their relationships with humans. For instance, pigs with access to leftover or refuse foods from humans tend to have higher $\delta^{15}N$ values than pigs that do not. Moreover, pigs that are suspected to have had access to such foods can have similar $\delta^{15}N$ values as in humans from the same area. Additionally, access to marine foods and/or agricultural crops will also impact the $\delta^{15}N$ and $\delta^{13}C$ values of pigs. The issue of whether certain foods were deliberately fed to pigs (i.e., whether pigs were domesticated and deliberately fed human refuse, or whether wild pigs opportunistically scavenged from human communities) is not straightforward, despite claims to the contrary in some studies.

Research objectives

In this study, pigs from Liang Bua (Flores, Indonesia) are examined using nitrogen and carbon stable isotopes in order to explore possible changes to their diet and/or environment over the past ~5,000 years. This temporal period is of particular interest because major changes occurred at Liang Bua during this time and these changes are thought to be related to the dispersal of Austronesian people and/or culture (Bellwood, 2005, 2017; Morwood et al., 2009; Sutikna et al., 2018; Tocheri et al., 2020). For instance, human populations in this area transitioned from a foraging way of life to a sedentary one based on farming, as evidenced by the first appearance of pottery within the Liang Bua stratigraphic sequence ~3 ka (Sutikna et al., 2018; Julianto et al., 2020). Moreover, previous preliminary studies of $\delta^{13}C$ in modern humans, pigs, and other animals at the site have shown that a noticeable shift to including C_4 plants in the diet began ~2,700 years ago (Anderson, 2011; Munizzi, 2013; Tocheri et al., 2020). However, pig remains at Liang Bua suddenly increased in abundance ~3,600 years ago, after having been relatively scarce earlier in the Holocene and lacking any evidence of pigs on Flores prior to that (van den Bergh et al., 2009; Sutikna et al., 2018, 2020). Pigs were almost certainly brought to the island intentionally by people, either through trade or population dispersal (Larson et al., 2007; Morwood et al., 2009; van den Bergh et al., 2009; Sutikna et al., 2018, 2020), but this appears to have happened before pottery and other non-endemic animals (e.g., macaques, civets, porcupines, chickens) were introduced (Sutikna et al., 2018, 2020; Julianto et al., 2020; Evans et al., 2020a,b; Meijer et al., 2020). By documenting the variation and possible changes in $\delta^{15}N$ and $\delta^{13}C$ values of pigs through time at Liang Bua, this research aims to shed light on how people managed these animals as a resource during this interesting period. Most importantly, this research attempts to address whether pig diet changed in relation to the onset of farming activities in the area surrounding Liang Bua ~3 ka and, if so, in what ways.

Materials and Methods

Samples

Fifty-three pig bones and 27 pig teeth were selected for stable isotope analysis. All of these samples were derived from Sector XXV, a 2 x 2 m excavation near the eastern wall of Liang Bua (Figure 1). Age-depth models for this Sector, based on 15 calibrated radiocarbon ages from charcoal (Sutikna et al., 2018), suggest that these samples range in age between 4,292 and

430 years old (Table 1). These samples were combined with previously obtained isotopic data from 8 bone and 9 tooth pig samples from Sector XVI, another 2 x 2 m excavation near the eastern wall of the cave (Munizzi, 2013). Age-depth models for Sector XVI, based on 21 calibrated radiocarbon ages from charcoal (Sutikna et al., 2018), suggest that these samples range in age between 3,631 and 589 years old (Table 1). One horse and seven human samples were also selected, to provide a comparative context for interpreting the pig stable isotope data (Table 2). These samples derive from various excavated Sectors (Figure 1) and all but one (sample 98) were directly radiocarbon dated at the Oxford Radiocarbon Accelerator Unit in University of Oxford's Research Laboratory for Archaeology and the History of Art (RLAHA). The human samples range in age between 4,292 and 2,596 cal. years BP, while the horse's age is 241 cal. years BP.

Collagen extraction

The collagen extraction procedures for all the pig samples and one of the human samples (sample 98) used in this study follow those of Longin (1971) with modifications by the Laboratory for Stable Isotope Science at the University of Western Ontario, Canada (Munizzi, 2013). Collagen extractions were performed at the University of Central Florida in the laboratory of Dr. Tosha Dupras. Initially, all of the samples were cleaned with distilled water in an ultrasonicator to remove unwanted attached materials. The samples were then dried in an oven at 90°C for 24 hours. Each sample was then soaked in 15 ml of 0.5 M HCl at room temperature of ~21°C. During the soaking process, the acid was changed when the pH increased higher than one, which occurred every day for approximately the first week. After that, the acid was changed every two or three days, depending on the pH of each sample, until all unwanted material was dissolved leaving only the collagenous isomorph. Next, the samples were rinsed with distilled water and soaked in 15 ml of 0.10 M NaOH at room temperature of ~21°C for 20 minutes to remove the humic and fulvic acids. This process was repeated six times or more until the sample colour remained clear. Each sample was then rinsed several times in 15 ml of 0.25 M HCl and then distilled water with added HCl (pH 3.0) was added to bring the sample pH back to 2.5–3.0. Samples were then placed in a 90°C oven for ~16 hours to solubilize (gelatinize). Gelatinized samples were pipetted into two-dram glass vials and placed in an oven at 90°C until the remaining collagen was dry. Collagen extraction for the remaining seven comparative samples (6 human, 1 horse) was conducted at University of Oxford's RLAHA using a modified version of the Longin (1971) method, which is outlined in Richards and Hedges (1999) (see also Alagich et al., 2018; Fernández-Crespo et al., 2020).

Stable isotope measurements

The 32 pig bone, 10 pig tooth, and 1 human tooth samples from Sector XXV that preserved 1% or more collagen (by bone weight) were analyzed at the Light Stable Isotope Mass Spec Lab at the University of Florida using a Thermo Delta V Advantage isotope ratio mass spectrometer with a ConFlo IV interface attached to a Thermo IsoLink elemental analyzer in continuous-flow mode. For all samples, the weight percentages of carbon (wt% C) and nitrogen (wt% N), and the raw δ^{13}C and δ^{15}N values were measured and the C:N ratios (atomic) were calculated. The δ^{15}N and δ^{13}C values were drift corrected and calibrated to the AIR and VPDB scales, respectively, using a two-point linear calibration anchored by USGS41 (δ^{13}C = +37.63‰, δ^{15}N = +47.57‰) and USGS40 (δ^{13}C = –26.39‰, δ^{15}N = –4.52‰). Additional USGS40 (i.e., not included in the calibration curve) was used as a check standard. Seven samples (6 pig, 1 human) were analyzed in duplicate to assess the accuracy and precision of the isotope measurement results. The standard uncertainty of these pig isotopic data was calculated following recommendations proposed by Szpak et al. (2017) using the calibration and check standards mentioned above as well as the sample duplicates. In combination with the seven sample duplicates, the calibration and check standards resulted in a standard uncertainty for δ^{15}N of 0.31‰ and for δ^{13}C of 0.15‰.

The 17 samples from Sector XVI previously reported by Munizzi (2013) were analyzed at the Smithsonian Institution's Stable Isotope Laboratory using a Thermo Scientific Delta V Advantage mass spectrometer coupled with a Costech ECS 4010 elemental analyzer via a Conflo IV gas interface. The δ^{15}N and δ^{13}C values from Sector XVI were drift corrected and calibrated on the AIR and VPDB scales, respectively, using two-point linear calibration against two in-house reference materials, Acetanilide B and Urea-UIN3. Measurement of uncertainty was monitored using Keratin, an in-house check standard. Precision for δ^{15}N values was ±0.22‰ or less and precision for δ^{13}C values was ±0.17‰ or less, based on repeated measures of calibration and check standards (Christine France, personal communication, 2020).

The remaining seven comparative samples (6 human, 1 horse) were analyzed at University of Oxford's RLAHA using a SER-CON 20/22 isotope ratio mass spectrometer coupled with an elemental analyzer in continuous flow mode. Five of these samples (4 human, 1 horse) were analyzed in duplicate and were calibrated using a two-point calibration anchored by USGS40 and USGS41. The other two human samples were ^{14}C dated and had approximate δ^{13}C and δ^{15}N values measured using a single-point calibration. Thus, these delta-values are not as accurate as those obtained using a two-point calibration. Unfortunately, there was not enough collagen remaining after ^{14}C dating to re-analyze these two samples for δ^{13}C and δ^{15}N. However, for both δ^{13}C and δ^{15}N, the five samples with values obtained from a two-point calibration and values associated with ^{14}C dating (i.e., one-point calibration) show a strong correlation (R^2 =

0.966 and 0.996, respectively) (Figure 2). Thus, the two human samples whose δ^{13}C and δ^{15}N values were measured in associated with ^{14}C dating are included in this thesis for comparison, but any interpretations based on these two samples should be made with caution. Further details about the drift correction, calibration, precision, and accuracy for these measurements are unavailable at this time but have been requested from University of Oxford's RLAHA.

Statistical analyses

To examine variation in Liang Bua pig δ^{15}N and δ^{13}C values through time, all samples were initially organized into two groups based on their temporal relationship to the earliest evidence of pottery at the site, which occurred ~3,000 years ago (Sutikna et al., 2018; Julianto et al., 2020). Assuming that pottery is a reasonable proxy for agriculture—and previous studies have shown that a dietary shift to C_4 occurred at Liang Bua in humans, rats, and pigs ~2,700 years ago (Anderson, 2011; Munizzi, 2013; Tocheri et al., 2020)—pig samples >3,000 years old were used to represent a Before Farming group (BF) and those <3,000 years ago were used to represent an After Farming group (AF). The BF group ranges in ages from ~4,300 and 3,000 years ago. To examine possible additional variation through time, the AF group was further divided arbitrarily into two groups spanning ~1,500-year intervals each (AF1: 3,000–1,500 years old; AF2: 1,500–400 years old). The stable isotope values of bones and teeth were also examined separately to see if any developmental signal was present in the stable isotope values of the samples.

All statistical analyses were conducted using PAST 4.04 software (Hammer et al., 2001) and for all statistical comparisons an alpha value of 0.05 was used. For all group comparisons, the data were first evaluated for normality (Shapiro-Wilk and Anderson-Darling tests) and homogeneity of the error variances (Levene's Test). A one-way ANOVA and Tukey's post hoc pairwise comparison test was used to test for statistical significance between group means if the assumptions of normality and homoscedasticity were not rejected. Alternatively, if one or both assumptions were rejected, then a Kruskal-Wallis test was used to test for significance between group means and a Mann-Whitney U test was used for all post hoc pairwise comparisons.

Results

Collagen extraction

Collagen preservation is critically important for accurate dietary reconstructions using stable isotopes. Ambrose (1993) suggests that 1% or more of collagen by weight can be considered well-preserved enough for dietary reconstruction. Of the 80 new pig specimens sampled for this study, 38 had collagen yields less than 1% or (in a few instances) a problem with the collagen extraction procedure that resulted in insufficient sample. The remaining 42 samples had collagen yields of more than 1% and were sent for isotopic measurement on the mass spectrometer. Figure 3 shows that there was a weak negative linear relationship ($y = -124.54x + 2848.2$, $r = -0.52$, $R^2 = 0.27$) between the collagen yield and age of each sample. All 16 samples that had collagen yields greater than 9% were less than ~1,600 years old. Overall, the collagen yields for samples less than 2,000 years old ranged from 0% to 20.8%. For samples between 3,000 and 2,000 years old, collagen yields ranged from 0% to 8.6%. Finally, collagen yields decreased again, on average, for samples older than 3,000 years old with a range of 0% to 5.3%. Moreover, 10 of 30 samples older than 3,000 years old and 20 of 65 samples that were younger than 3,000 years old were excluded due to the lack of sufficient collagen yield (i.e., <1%).

Collagen preservation was assessed further using the C:N ratio (atomic) (Schoeninger et al., 1989; Wright and Schwarcz, 1999), with a range between 2.9 and 3.6 considered acceptable as this suggests that the isotopic composition of the collagen was not altered significantly by the taphonomic environment (Ambrose, 1993; DeNiro et al., 1985). Based on values observed in fresh bone collagen, well-preserved samples also typically should have wt% C of about 15–47% and wt% N of 5–17% (Ambrose, 1990). Alternatively, based on 2,146 collagen samples from the Oxford ^{14}C database, van Klinken (1999) suggests that the wt% C and wt% N should have ranges of 26–43% and 11–16%, respectively.

Table 3 shows the collagen yields, C:N ratios (atomic), wt% N, and wt% C for all of the pig samples and their duplicates. Three samples (22, 41A, and 53), one of which was a duplicate, fell outside of the acceptable range for the C:N ratio and were therefore excluded from further analysis. No clear relationship (Figure 4) was observed between the collagen yields and the C:N ratios (atomic) for the total sample. Twenty-nine samples had wt% C and wt% N values outside of the acceptable ranges proposed by van Klinken (1999). Of these samples, six (two of which were duplicates) had slightly higher wt% C (samples 1, 12, 43, 44A-B, 46A-B, 51), five (two of which were duplicates) had lower wt% C (samples 23A, 36, 39, 40, 41B), 16 (three of which were duplicates) had lower wt% N (samples 23A-B, 27, 28, 29A-B, 30, 32, 35–40, 41B, 42, 54, 59), and two (one of which was a duplicate) had slightly higher wt% N (samples 43, 44A-B) (Table 3). However, all but one of these samples (sample 41B) still fell within the acceptable wt% C and wt% N ranges suggested by Ambrose (1990) and all had acceptable C:N ratios along with stable isotope values that fell within the ranges of the rest of the sample. Therefore, all of these samples were included in this study for further analysis.

Isotopic results

As mentioned above, one of the duplicate samples (sample 41A) was excluded from further analysis due to its C:N ratio. All of the remaining duplicate samples had resulting measurements that were broadly similar to their respective original samples, thus the measured stable isotope values for each of these were averaged for further analysis. This resulted in a total of 57 (39 bone, 18 tooth) pig specimens with stable isotope values, 16 (eight bones, eight teeth) of which derived from Munizzi (2013). All final calibrated δ^{13}C and δ^{15}N values are shown in Table 3.

The total stable isotope data set for Liang Bua pigs, whose ages spanned from ~4,300 until ~400 years ago, had a δ^{15}N range of +3.8‰ to +9.4‰ and a δ^{13}C range of –9.6‰ to –21.3‰. An initial comparison was conducted to test whether there was a 'nursing effect' with higher δ^{15}N values in the bones and teeth of immature pigs who may have been consuming milk from their mothers (Reynard and Turros, 2015). Although the range of δ^{15}N values in pig teeth (+6.0‰ to +9.4‰) was smaller than that observed for pig bones (+3.8‰ to +9.4‰), the four deciduous teeth did not show higher δ^{15}N values compared to those of permanent teeth (Figure 5). In the Before Farming (BF) group, one (sample 34) out of four teeth was deciduous and had a δ^{15}N value of +8.1‰, while the three permanent teeth had δ^{15}N values of +9.1‰ to +9.4‰. In the After Farming (AF) group, there were three deciduous teeth (samples 2, 3, 6) with δ^{15}N values of +8.3‰, +6.7‰, and +6.0‰, whereas six permanent teeth had δ^{15}N values of +6.8‰ to +7.9‰. Therefore, the δ^{15}N values of all the deciduous teeth in this study did not show any enrichment due to potential effects of milk-consuming. For bones, a single juvenile pig bone (sample 42) from the BF group had a relatively high δ^{15}N value of +9.3‰. However, this value was still within the range (+7.5‰ to +9.4‰) observed for adult bones from the BF group. Based on this evidence, the stable isotope values from the deciduous teeth and single juvenile bone sampled in this study should not skew the temporal comparisons. Thus, all bone and tooth samples (juvenile and adult) were pooled for further analyses.

The BF group consisted of 15 specimens that yielded a mean δ^{15}N value of +8.5‰ (range = +7.5‰ to +9.4‰) and a mean δ^{13}C value of –20.1‰ (range = –20.7‰ to –19.3‰ (Figures 6–9). In contrast, the After Farming (AF) group consisted of 42 specimens that yielded a mean δ^{15}N value of +7.2‰ (range = +3.8‰ to +9.3‰) and a mean δ^{13}C value of –17.3‰ (range = –21.3‰ to –9.6‰). The variances of both δ^{15}N and δ^{13}C were not homogenous and the normality tests also indicated that the data were not normally distributed. Thus, the nonparametric Kruskal-Wallis test was used and resulted in significant differences between the BF and AF groups for δ^{15}N, $H(1, 56) = 13.94$, $p < 0.001$, and δ^{13}C, $H(1, 56) = 17.91$, $p < 0.001$.

When AF was divided into AF1 and AF2 groups, AF1 consisted of 16 specimens that yielded a mean δ^{15}N value of +7.4‰ (range = +3.8‰ to +9.3‰) and a mean δ^{13}C value of –18.6‰ (range –21.2‰ to –15.7‰). AF2 consisted of 26 specimens that yielded a mean δ^{15}N value of +7.1‰ (range = +4.2‰ to +9.3‰) and a mean δ^{13}C value of –16.5‰ (range = –21.3‰ to –9.6‰). For the comparison of δ^{15}N values between BF, AF1, and AF2, the assumptions of the ANOVA model were not rejected. The null hypothesis that the means of these groups were the same was rejected, $F(2, 54) = 7.25$, $p < 0.05$. Tukey's HSD multiple comparison test showed that there were significant differences in mean δ^{15}N between BF and AF1, $Q(1, 30) = 3.723$, $p=0.029$, and between BF and AF2, $Q(1, 40) = 5.322$, $p<0.001$. However, there was no significant difference in mean δ^{15}N between AF1 and AF2, $Q(1,41) = 1.219$, $p=0.666$. For δ^{13}C, the assumptions of the ANOVA model were rejected and the nonparametric Kruskal-Wallis test was used instead. Results showed that there was a significant difference in δ^{13}C between BF, AF1, and AF2, $H(2, 54) = 19.78$, $p<0.001$. Mann-Whitney pairwise tests showed that there were significant differences in mean δ^{13}C between BF and AF1, $U(1, 30) = 37.5$, $p<0.001$, and between BF and AF2, *U*(1, 40) = 44, $p<0.001$. However, there was no significant difference in mean δ^{13}C value between AF1 and AF2, *U*(1, 41) = 143, p=0.1.

Discussion

At Liang Bua, statistically significant shifts in pig carbon and nitrogen isotope compositions occur after the first appearance of pottery at the site ~3,000 years ago (Sutikna et al., 2018; Julianto et al., 2020). For δ^{15}N, the mean value in pigs decreased from +8.5‰ (BF) to +7.2‰ (AF) while for δ^{13}C, the mean value in pigs increased from –20.1‰ (BF) to –17.3‰ (AF). There were no significant differences in mean stable isotope values between AF1 (~3,000–1,500 years) and AF2 (~1,500–400 years), although some subtle differences between these two groups in their ranges of values were observed (Figures 6–9). AF1 and AF2 were also each significantly different from the BF group for both δ^{15}N and δ^{13}C.

The main question that must first be considered is if, and to what degree, these temporal differences in δ^{13}C and δ^{15}N are related to changes in Liang Bua pig diets. Although pigs with pure C_3 diets are expected to range in δ^{13}C from –31‰ to –16‰ due to the full range of δ^{13}C values in C_3 plants, the more enriched or higher values should usually only occur in extremely arid climates, which is not the case on Flores. Instead, if the BF range of δ^{13}C is taken as a baseline for local C_3-based diets, it is evident that there is a shift in δ^{13}C values in the AF groups that is driven at least in part by introduction of C_4 plants to pig diets, as Liang Bua pigs with δ^{13}C values greater than –19‰ clearly have a C_4 component in their diet. For δ^{15}N, however, the relationship to animal diet is more complicated as a number of different factors in addition to diet, such as the source(s) from which plants are obtaining nitrogen and levels of aridity/precipitation, may be contributing to the isotopic variation (Ambrose, 1993; Schwarcz et al., 1999; Szpak et al., 2013; Pate and Anson, 2018; Metcalfe and Mead, 2019). Because of these differences between δ^{13}C and δ^{15}N, they are each discussed separately in the following sections.

Changes through time in Liang Bua pig δ^{13}C values

Overall, the δ^{13}C values from all Liang Bua pig samples indicate considerable variation in the proportions of C_3 and C_4 resources that were consumed (Figures 8 and 9). Out of 57 pig samples, 32 (56.1%) ranged from –21.3‰ to –18.9‰ (no definitive evidence of C_4 -plant consumption), 7 (12.3%) ranged from –12.7‰ to –9.6‰ (primarily a C_4-based diet), and 18 (31.6%) ranged from –19.1‰ to –15.7‰ (a mixed C_3–C_4-based diet). Pigs in the BF group displayed a δ^{13}C range of –20.7‰ to –19.3‰ with a mean of –20.1‰, suggesting a diet based primarily on C_3 resources. In comparison, four human samples from this same temporal interval displayed slightly higher values that were still within the C_3 range (δ^{13}C range of –19.8‰ to –18.7‰, mean of –19.3‰). These values indicate that C_3 resources were the main component of the 'before farming' human diet at Liang Bua. The slight enrichment in human δ^{13}C over pig values is likely a trophic effect.

In contrast to the BF group, pigs in the AF1 group displayed a δ^{13}C range of –21.2‰ to –15.7‰ with a mean of –18.6‰ and those in the AF2 group displayed a δ^{13}C range of –21.3‰ to –9.6‰ with a mean of –16.5‰. 'After farming' pigs with δ^{13}C values at the lower end of these ranges clearly had C_3-based diets, whereas AF2 pigs with values at the uppermost end of the range mostly consumed C_4 foods and some pigs in the AF1 group had a mixed C_3–C_4-based diet. The increase in available C_4 resources is also indicated by three human samples from the AF1 interval and a horse sample from the AF2 interval. The horse sample had a δ^{13}C value of –12.3‰ suggesting it had a mostly C_4-based diet. The human samples displayed a δ^{13}C range of –15.5‰ to –15.0‰ with a mean of –15.3‰ suggesting a mixed C_3–C_4-based diet and like in the previous human samples, perhaps a slight trophic enrichment effect from consuming pig or other animal protein (Richards et al., 2002, 2006; Fuller et al., 2006). These human samples from the AF1 interval are particularly important as they are directly dated to 2,700–2,600 cal. years BP and represent the earliest burials at Liang Bua that include pottery and polished adzes as grave goods (Julianto et al., 2020) as well as show a portion of Austronesian genetic ancestry (Oliveira et al., 2020).

In combination, these data support the hypothesis that the appearance of pottery at Liang Bua ~3,000 years ago signals the onset or intensification of farming activities in this area and the observed $\delta^{13}C$ values suggest that these activities included one or more domesticated C_4 plants (Anderson, 2011; Munizzi, 2013; Sutikna et al., 2018; Julianto et al., 2020; Tocheri et al., 2020). The most likely candidate for a domesticated C_4 plant grown around Liang Bua at that time is foxtail millet (*Setaria italica*) (Anderson, 2011). The earliest evidence of domesticated foxtail millet occurs in China as early as 11 to 10 thousand years ago in the North China Plain (Yang et al., 2012) and is also documented ~8,700 cal. years BP at Cishan Neolithic sites (Lu et al., 2009). Foxtail millet and rice are thought to have been the primary agricultural crops of Austronesian people, who dispersed from Taiwan ~5,000 years ago and spread across Island Southeast Asia and beyond over the next several thousand years (Bellwood, 2005, 2017). Almost all languages spoken throughout Indonesia today, including those on Flores, are part of the Austronesian language family and the relationships among these languages are considered by many to be evidence of this last major prehistoric dispersal of modern humans (Gray et al., 2009; Bellwood, 2017).

Changes through time in Liang Bua pig $\delta^{15}N$ values

There are several factors that could impact Liang Bua pig δ^{15}N values though time in addition to potential changes in diet. In particular, increased aridity and decreased precipitation could increase the δ^{15}N values of plants at the base of the food web with cascading effects for primary and secondary consumers (Schwarcz et al., 1999), whereas high precipitation could decrease the δ^{15}N values in the plants at the base of the food web (Stevens and Hedges, 2004). Griffiths et al. (2009) reported that the mean annual rainfall at Liang Luar (a cave located approximately 600 m from Liang Bua) is 1,200 mm, of which ~828 mm falls between December and March (the summer monsoon season) and only ~60 mm between June and September (the winter dry season). Using oxygen stable isotopes (δ^{18}O) in a stalagmite from Liang Luar, Griffiths et al. (2010) reconstructed the paleoclimate during the Holocene on Flores. Speleothems such as stalagmites and flowstones are excellent proxies for terrestrial paleoclimate due to their sensitivity in climate variability and can be dated with high precision uranium-series dating (Griffiths et al., 2010). The Liang Luar stalagmite's detailed record spanning 12,070 to 360 years ago suggested that although higher rainfall from the Australian-Indonesian Summer Monsoon occurred until ~6,000 years ago, rainfall levels have remained relatively stable and similar to present levels for the past 6,000 years (Griffiths et al., 2010). Moreover, the combined records of fluid-inclusion ($\delta^{18}O_f$) and calcite ($\delta^{18}O_c$) suggest that the air temperature in Liang Luar averaged ~24°C and has been relatively stable over the past ~10,000 years (Griffiths et al., 2010).

Given the close proximity and similar altitude of Liang Luar and Liang Bua, it is reasonable to assume that Liang Bua experienced similarly stable precipitation and air temperatures over the past 6,000 years. Therefore, there is no evidence of extreme aridity or wetness that would systematically increase or decrease the δ^{15}N values of the plants and animals around Liang Bua during this temporal interval. Extensive landscape modification by humans, such as the clearing of forest for settlement or burning and manuring of areas for agriculture can have significant effects on the δ^{15}N cycle and composition of soil, plants, and animals (Szpak, 2014; Guiry et al., 2018; Guiry et al., 2020). However, there is no evidence for major deforestation or industrial-type agriculture on Flores until the present day. For these reasons, changes in pig δ^{15}N values through time at Liang Bua were likely driven primarily by variations in pig diet (Figures 6 and 7).

The horse sample from the site yielded a δ^{15}N value of +4.1‰, and two pig samples (samples 8 and 29) had similarly low values (Table 3, Figure 6). Although limited, these data suggest that at Liang Bua, an animal with a completely herbivorous diet might have a δ^{15}N value around +4‰. In comparison, the seven human samples had a mean δ^{15}N value of +10.1‰ and all but one of these were higher than observed for pigs during the past 5,000 years. The δ^{15}N of the one human sample (~2,600 cal. years BP) that fell within the whole-sample pig δ^{15}N range was also almost +1‰ higher than pigs from the same period (Figure 6). This suggests that humans at Liang Bua always had a higher trophic level than pigs at the site at any given time in the past. It is very likely that humans regularly incorporated pig meat or other animal protein into their diets.

Pigs from both AF groups had statistically significant lower mean δ^{15}N values and wider ranges of variation than did pigs from the BF group. The δ^{15}N ranges of all three groups extended as high as +9‰, but it is only in the AF groups that the ranges extended below +7‰. The magnitude of these differences suggest that Liang Bua pigs consumed diverse diets that spanned multiple trophic levels. The increased variability in AF pig δ^{15}N values may represent an increase in pig diet breadth or perhaps more likely, greater variability in the diets of individual pigs (e.g., specialization on particular foods). Potential implications of these results are explored further in the sections below.

Wild versus domesticated pigs?

The isotopic data for the Liang Bua pigs suggests a change in pig diets occurred after the onset of farming, but the AF pig samples may represent some combination of domesticated and wild pigs. Indigenous Manggarai people who live around Liang Bua today regularly make use of both wild and domesticated pigs which live in the forests around their villages (Benyamin Tarus, Romanus Jehaman, and Stanis Mbembak, personal communication, 2020). These wild pigs live in the forest during the dry season but appear more often around human villages and use local caves for shelter during the rainy season. Manggarai informants describe the diet of wild pigs as opportunistically omnivorous, with typical preferred foods including wild foxtail millet, coconut, palm fruit, and even the giant rat (*Papagomys armandvillei*). Domesticated crops also attract these wild pigs, especially corn, sweet potato, and peanuts. During the harvest season, wild pigs always become more abundant around the crop fields. For this reason, local people must monitor their farms 24 hours per day in order to protect their crops from wild pigs. They usually place wire traps around their crops to catch these wild pigs and they actively hunt them often. Successful traps and hunts help protect the crops while also providing an excellent source of animal protein that is shared equally among the people that participated.

Interestingly, Manggarai people do not try to domesticate these wild pigs. According to them, wild pigs cannot be domesticated and will constantly try to break their cages or enclosures and escape. Instead, local people obtain their domesticated pigs by buying or trading for them. Usually, they will buy a couple of domesticated pigs and then attempt to breed them. For

Manggarai people, domesticated pigs are closely integrated into their culture, especially their traditional ceremonies. For example, there are at least three ceremonies (harvest, wedding, and funeral) that require pig meat for the main supper and usually involve the slaughter of three to five pigs. The Manggarai feed their domesticated pigs a mixture of mostly C_3 plants that usually consists of rice or corn bran, banana trunk, cassava, cassava leaves, sweet potatoes, sweet potato leaves, and *ampas tahu* (the fibrous residue left over from tofu processing). If they have enough rice, then they will also add it to this mixture. These various ingredients are typically mixed together with water and thus local people do not usually provide additional water for the pigs to drink. In addition, they will also often give their domesticated pigs leftover food that can include meat in addition to vegetables and grains from their household.

Similar wild pig behaviours and human pig-management practices also occur elsewhere in Indonesia. On Sulawesi, the island directly north of Flores, wild pigs are abundant and known to eat coconuts and tubers and will even consume shellfish when they live near mangrove forests (Suryanto[1], personal communication, 2020). In terms of human crops, wild pigs like to eat corn and sweet potatoes and local people do not attempt to domesticate these wild pigs, instead they hunt and trap them for meat. Domesticated pigs are kept confined to small cages and fed sweet potato leaves mixed with rice bran, and occasionally leftover food from the household (Yanirsa Abigael, personal communication, 2020).

In Maluku, an island located northeast of Flores, wild pigs prefer to eat ripe coconuts, which are cracked open with the pig's teeth after they have fallen from the tree, and people will use ripe coconut as a lure when hunting wild pigs (Michael Lahallo, personal communication, 2020). Cassava is also a preferred food for these wild pigs and local people build traps to protect

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[1] Note it is common for Indonesian people to have only one name. Family names are used rarely and even for Indonesian people with more than one name, these are not equivalent to first and last names in Western societies.

their crops from pigs. In this part of eastern Indonesia, sago (*Metroxylon sagu*) is a major staple food for people and is their main source of carbohydrates. The production of sago requires multiple steps that usually take place in the forest where the sago tree grows and wild pigs are known to consume the food waste that results from the production process. In contrast, domesticated pigs are fed banana trunks, combined with rice bran, *ampas tahu*, and other leftover foods.

These examples underscore the complex relationships in eastern Indonesia between humans and both wild and domesticated pigs. Domesticated crops are clearly not only accessible to domesticated pigs. In some cases, wild pigs may have more access to particular crops than domesticated ones. For these reasons, pig domestication should probably not be inferred directly from changes in isotopic values only, and any inferences should be made with caution.

Liang Bua pig diet during prehistory

At Liang Bua, pigs gained access to more C_4-derived resources after the onset of farming (i.e., AF pigs had more enriched $\delta^{13}C$ values). At the same time, some AF pigs had much lower $\delta^{15}N$ values, which suggests less access to animal-based protein (Figure 10). These changes in pig $\delta^{13}C$ and $\delta^{15}N$ associated with farming differ from those observed in Norway at Skipshelleren, where the average $\delta^{13}C$ and $\delta^{15}N$ values of pigs remained similar before and after farming with only a slight increase in mean $\delta^{15}N$ from +3.8 (range = +3.1 to +5.3‰) to +4.4‰ (range = +2.5 to +5.9‰) (Halley and Rosvold, 2014). Although not formally tested in Halley and Rosvold (2014), an ANOVA analysis comparing pigs before and after farming indicates that neither their $\delta^{15}N$ nor their $\delta^{13}C$ values significantly differed [$F(1,18) = 2.494$, p=0.13 and $F(1,18) = 0.3315$, p=0.57, respectively]. This suggests that pigs from both before and after agriculture was introduced at Skipshelleren consumed similar foods.

The Neolithic site of Xipo, dated to between 6,000 and 5,500 years ago in China, offers a more useful comparison with the results from Liang Bua. At this site, two pigs and one dog had higher $\delta^{13}C$ and lower $\delta^{15}N$ values than pigs, dogs, and other animals from other Neolithic sites in China (Pechenkina et al., 2005), which was interpreted as evidence for deliberately feeding these animals a C_4 dominated diet rich in millet (Pechenkina et al., 2005). The $\delta^{15}N$ values of these 'domestic' animals were similar to those observed in bovids and cervids from the nearby site of Kangjia (4,500 to 4,000 years ago) and there were small walled features uncovered at Xipo thought to be possibly pigsties, suggesting that these pigs may have been raised in confinement (Pechenkina et al., 2005). Unless confined, domesticated pigs will commonly scavenge or be given leftover food from humans (and wild pigs may also have opportunities to scavenge human refuse), thus their stable isotope values may approach those of humans (Pechenkina et al., 2005). In this case study, multiple lines of evidence, including higher $\delta^{13}C$ and lower $\delta^{15}N$ values, were indicative of pig domestication.

At Liang Bua, 75.5% of the pig samples (43 out of 57) had $\delta^{15}N$ values above +7‰ and approached the human values (Figures 6 and 10). This makes it impossible to confidently attribute these particular samples to either wild or domesticated groups. They could have been wild pigs with an omnivorous diet which may or may not have included human refuse, or they could have been domesticated pigs that were fed human leftovers or were allowed to range and forage more freely. For example, the Ta'a tribes who live in settlements in Morowali, Central Sulawesi allow their domesticated pigs to range freely and sometimes feed them mixed vegetables, rice bran, and leftover food (Ahmad Azhar, personal communication, 2020). Further zooarchaeological research on the Liang Bua pig assemblage would be helpful for addressing the proportions of wild versus domesticated pigs represented at the site.

However, 24.5% of the Liang Bua pig samples (14 out of 57) had δ^{15}N values between +4‰ and +7‰, and these low-^{15}N pigs were all in the 'after farming' groups. These samples are arguably the best candidates for representing domesticated pigs at Liang Bua. Their low δ^{15}N values are consistent with restricted access to animal-based protein, in contrast to other pigs from the same temporal intervals who had much higher δ^{15}N values consistent with consuming animal protein. Today, Indigenous Manggarai people do not usually allow their domesticated pigs to range freely and instead keep them confined in cages usually not much larger than the pig itself, feeding them entirely C_3 and predominantly plant-based diets. If this behaviour was also practiced in the past, or if the pigs were confined in larger enclosures that restricted or considerably reduced their access to animal foods, it could reasonably explain why these 14 pig samples had such relatively low δ^{15}N values. Further support for the idea that low pig δ^{15}N resulted from domestication and/or confinement can be derived from a previous study of δ^{13}C and δ^{15}N in rats from Liang Bua (Anderson, 2011). These rats showed a similar δ^{13}C trend through time as the pigs, but there were no changes through time in rat δ^{15}N. Rather, the mean rat δ^{15}N values and ranges of variation before and after farming were similar (+6.8‰, +3.9‰ to +9.7 ‰, versus +5.7‰, +3.1‰ to +8.7‰, respectively). Thus, dietary restriction mediated by humans seems a more likely explanation for decreased pig δ^{15}N than any change in wild resource availability.

It is interesting that although δ^{15}N values decreased and δ^{13}C increased among some Liang Bua pigs after the onset of farming, the individual pigs with low δ^{15}N values did not have high δ^{13}C values (Figure 10). If the lower δ^{15}N values represent protein restriction associated with confinement (as argued above), these results indicate that foxtail millet or other C_4 crops were withheld from these domestic pigs. In contrast, millet was more likely scavenged from agricultural fields by wild or semi-domesticated free-ranging pigs. Interestingly, all but one of the foods typically fed to domesticated pigs around Liang Bua today are C_3 plants (corn bran is the single exception) suggesting that C_4 crops grown in this area have a long history of being reserved for human consumption. Furthermore, crop raiding is common among wild pigs on Flores and other Indonesian islands today, as described above.

Of additional interest are seven pig samples from the AF2 group that had the most enriched $\delta^{13}C$ values of the entire pig sample, ranging from –12.7‰ to –9.6‰. Six of these values were even more enriched than observed in the horse sample (–12.3‰) from the same temporal interval, suggesting that these particular pigs consumed as much or even more C_4 resources than the horse. Compared to the horse with a $\delta^{15}N$ value of +4.1‰, however, these seven pigs all had relatively high $\delta^{15}N$ values (+7.2‰ to +9.3‰), suggesting a more omnivorous diet. There are several ways in which pigs may have obtained access to abundant C_4 resources along with an omnivorous diet.

First, it is possible that these pigs were domesticated and fed mainly with C_4 plants, but they were either allowed to range freely or were fed leftover human food (or waste) that included animal protein. However, this explanation implies two completely distinct domestication strategies were used at Liang Bua—restriction of animal protein and lack of access to C_4 plants among one group of pigs (low $\delta^{15}N$ and low $\delta^{13}C$) and access to C_4 plants and animal protein among another group of pigs (high $\delta^{15}N$ and high $\delta^{13}C$)—which seems unlikely. Second, since the $\delta^{13}C$ values of these seven pigs were so different from the rest of the sample, it is also possible that these animals were brought by humans from elsewhere. For example, an isotopic study of animals from the sites of Pacung and Sembiran in northern Bali, Indonesia interpreted markedly different isotope values of goats compared with humans, pigs, and dogs from the same sites as evidence that some goats were imported as a trading commodity (possibly from South Asia) around 2,000 years ago. At Liang Bua, it is premature to make a similar interpretation but it would be worthwhile to revisit this issue with further research using other isotopic proxies like hydrogen, oxygen, or strontium, which could potentially resolve whether any of the pigs were traded or imported from elsewhere. Third, the AF2 samples with high δ^{13}C and δ^{15}N values could have been wild pigs that were able to scavenge domesticated C_4 crops while also obtaining an animal-based protein component similar to most of the other Liang Bua pigs. This last explanation seems most likely, particularly if my interpretation of domestication as confinement with minimal protein and C_4 access is correct (as argued above).

Conclusion

This study has shown that the δ^{13}C values of pigs and humans changed at Liang Bua after the onset of farming ~3,000 years ago, as did the δ^{15}N values of pigs. Changes in δ^{13}C values suggest that beginning after ~3,000 years ago, one or more types of C_4 plants were consumed by pigs and humans, leading to more enriched δ^{13}C values compared with earlier populations. Foxtail millet, a C_4 plant long associated with the dispersal of Austronesian people throughout island Southeast Asia, is the most likely candidate for a C_4 plant used as an agricultural crop around Liang Bua during that time. Changes in the δ^{15}N values of pigs suggest that after ~3,000 years ago, the diet of some of the Liang Bua pigs was restricted, resulting in lower δ^{15}N values than observed among any pigs from earlier time intervals. Furthermore, these 'captive' pigs did not have access to the newly available C_4 resources. The custom of keeping domesticated pigs in small enclosures and provisioning them with mostly C_3 plants, practiced by Indigenous Manggarai people living around Liang Bua today, is the most likely explanation of these results.

This study highlighted the potential of stable isotope analysis for reconstructing ancient human and animal diets and animal husbandry practices in Indonesia. Stable isotope analysis is still relatively uncommon in Indonesian archaeology but should be made a regular component of further archaeological research in this country. This includes incorporating stable isotope analysis into plans for new archaeological excavations as well as research on existing collections that are curated at various institutions across Indonesia.

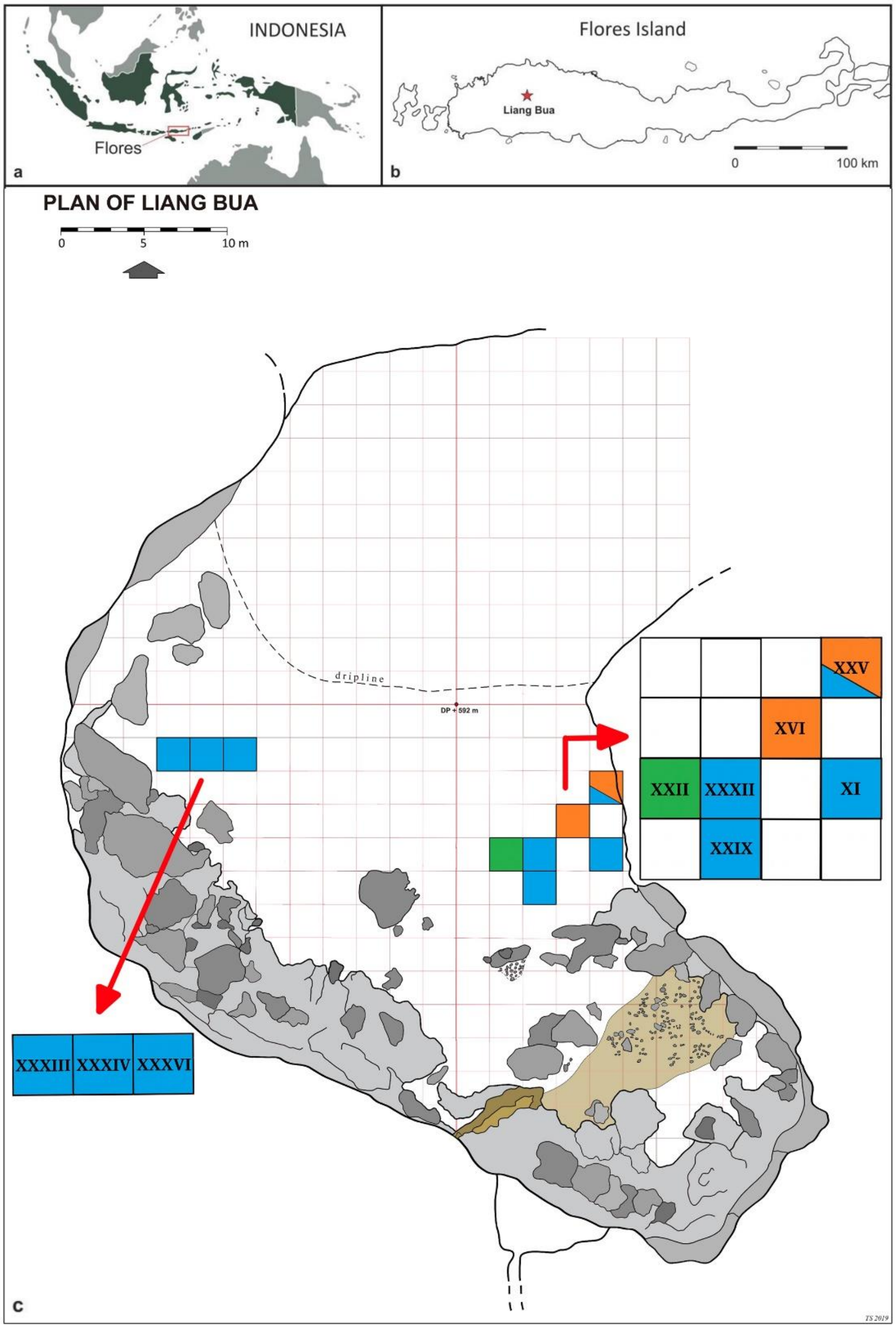

Figure 1. Location of Flores in Indonesia (a); location of Liang Bua on Flores (b); plan of Liang Bua (c) showing where the samples used in this study were excavated (pigs, orange; humans, blue; horse, green) (modified from Sutikna et al., 2016).

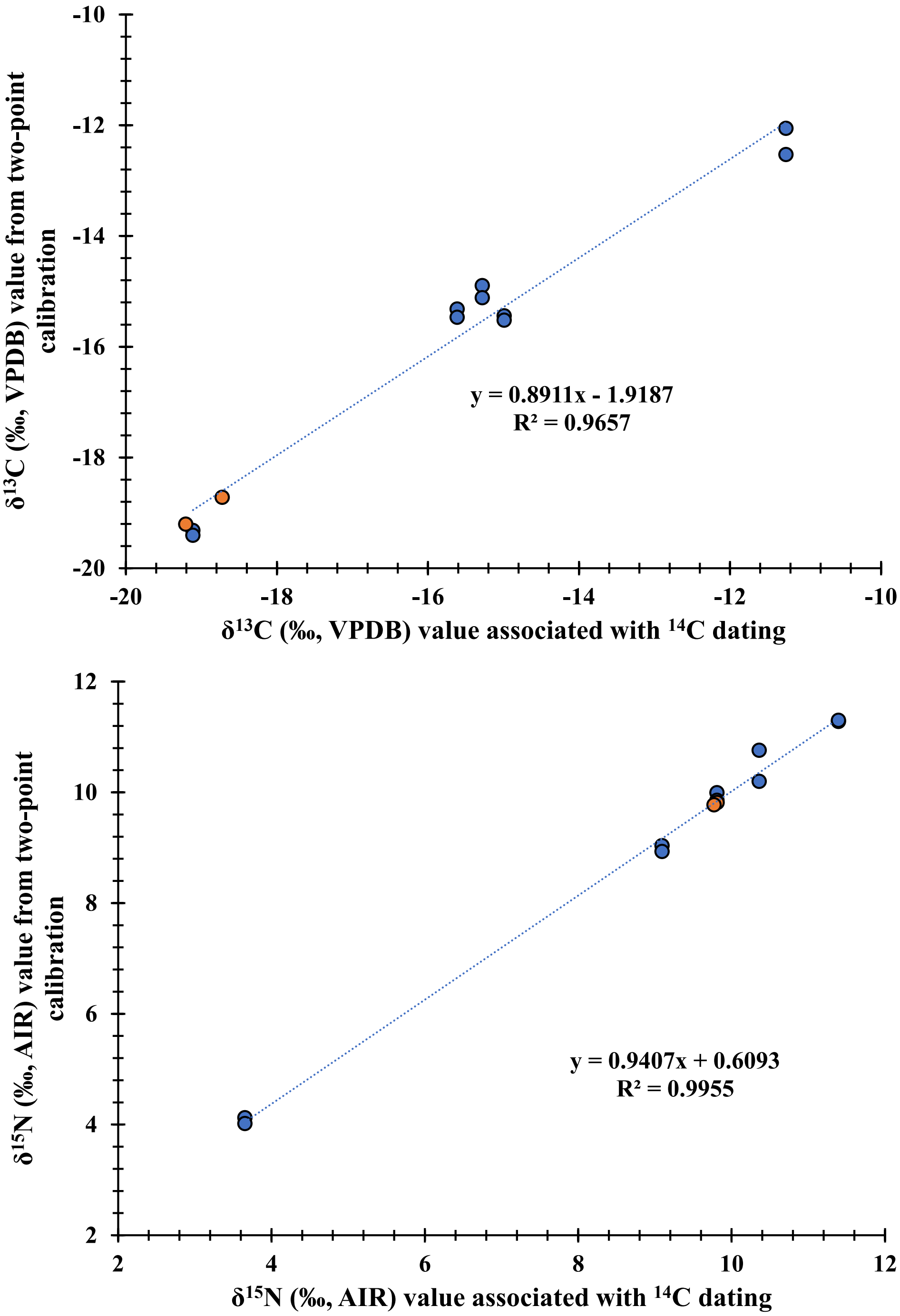

Figure 2. Linear relationships between the values from 2-point calibration and those obtained when the samples were ^{14}C dated (δ^{13}C, top; δ^{15}N, bottom) for the five samples (blue circles), which were run in duplicate for 2-point calibration method but only once for ^{14}C dating. The orange dots are the two human samples that lacked delta values obtained via a 2-point calibration. For those two samples, the same delta values are plotted on the x and y axis to demonstrate that the delta values associated with ^{14}C dating only are extremely similar to the values that would be expected from a 2-point calibration (i.e., they plot very close to the regression line).

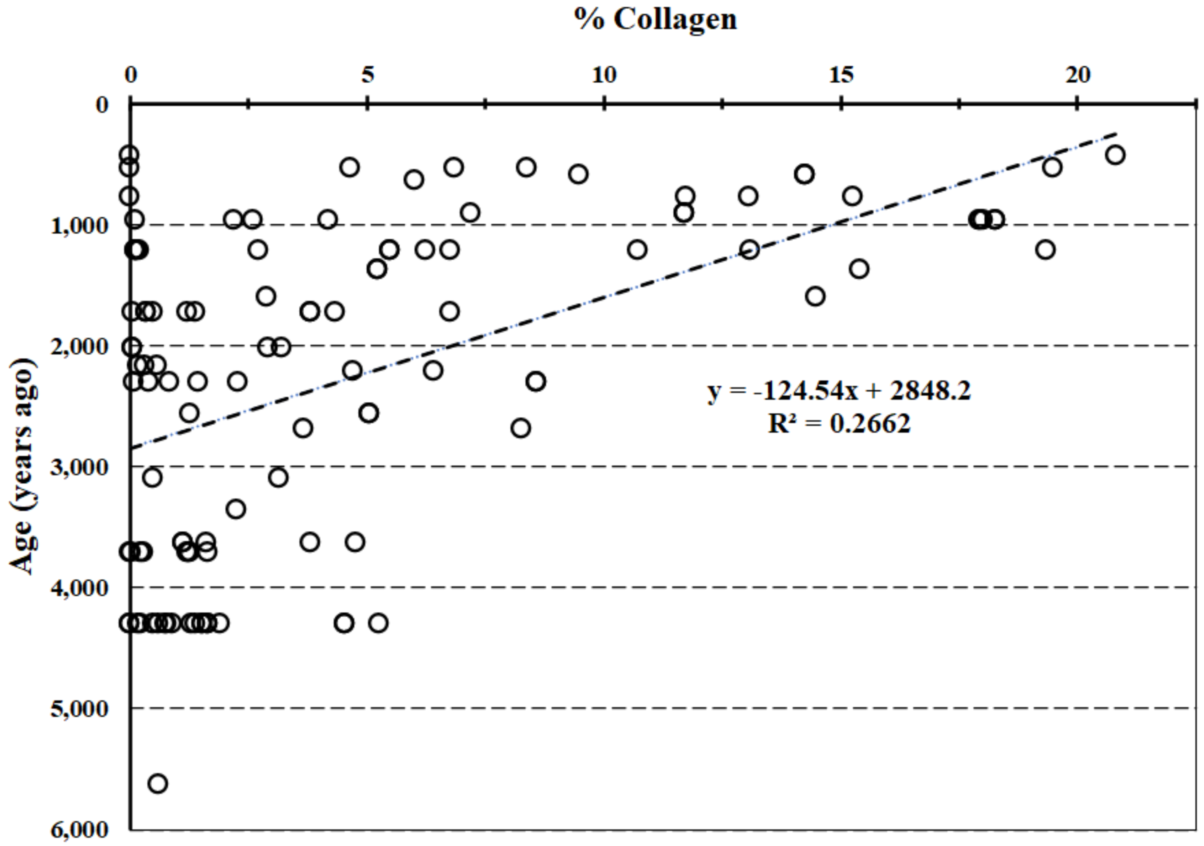

Figure 3. Linear relationship between collagen yield and age of each sample.

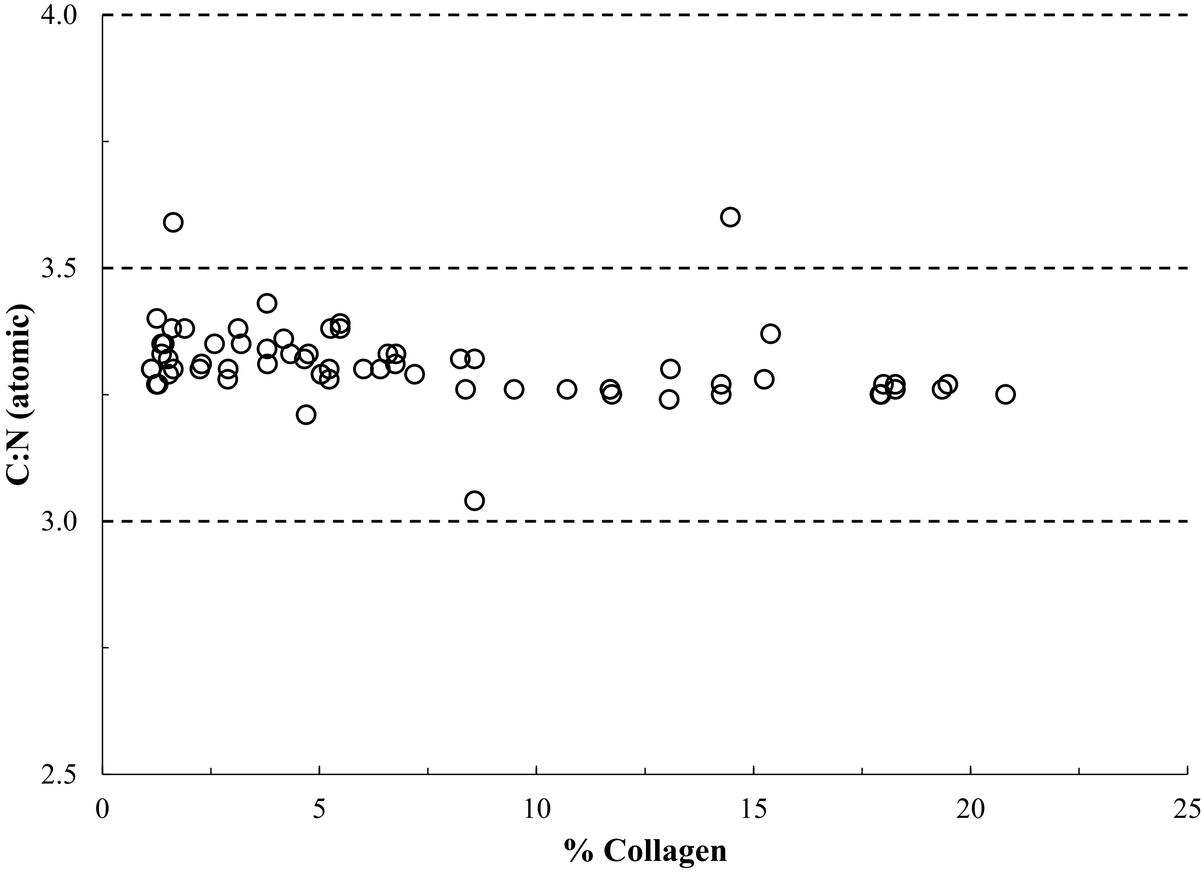

Figure 4. The relationship between collagen yield (%) and C:N ratio (atomic).

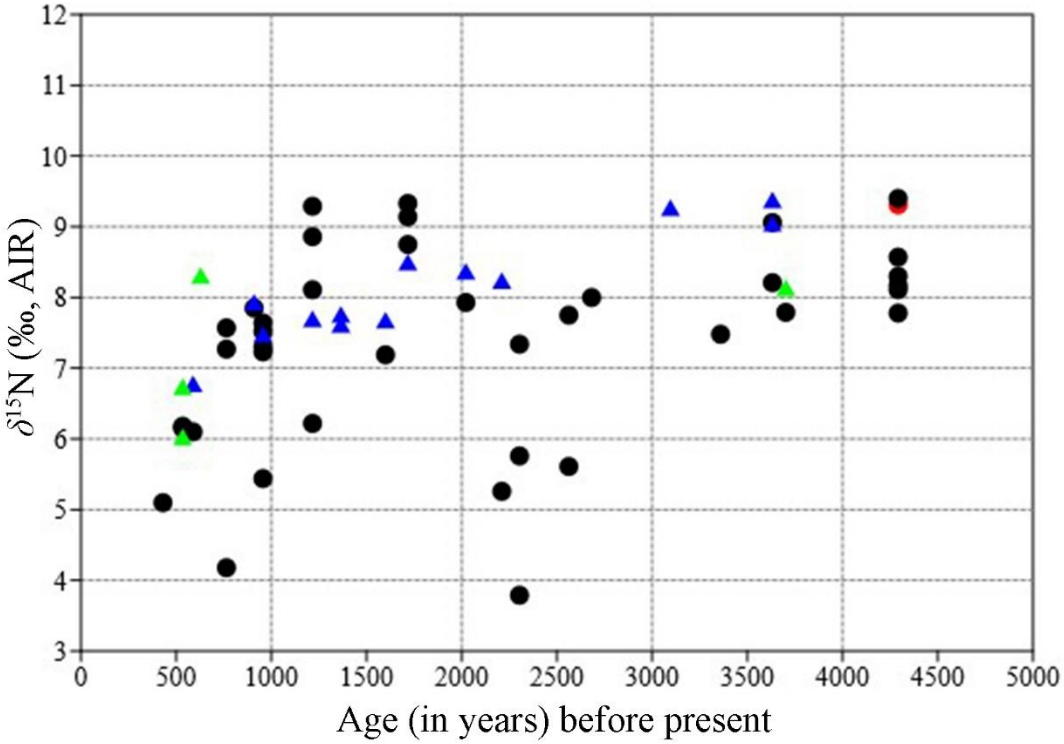

Figure 5. Scatter plot of δ^{15}N values for pigs in this study shows no particular trophic effect due to potential breastfeeding in juveniles (black circles, adult bones; red circles, juvenile bones; blue triangles, permanent teeth; green triangles, deciduous teeth).

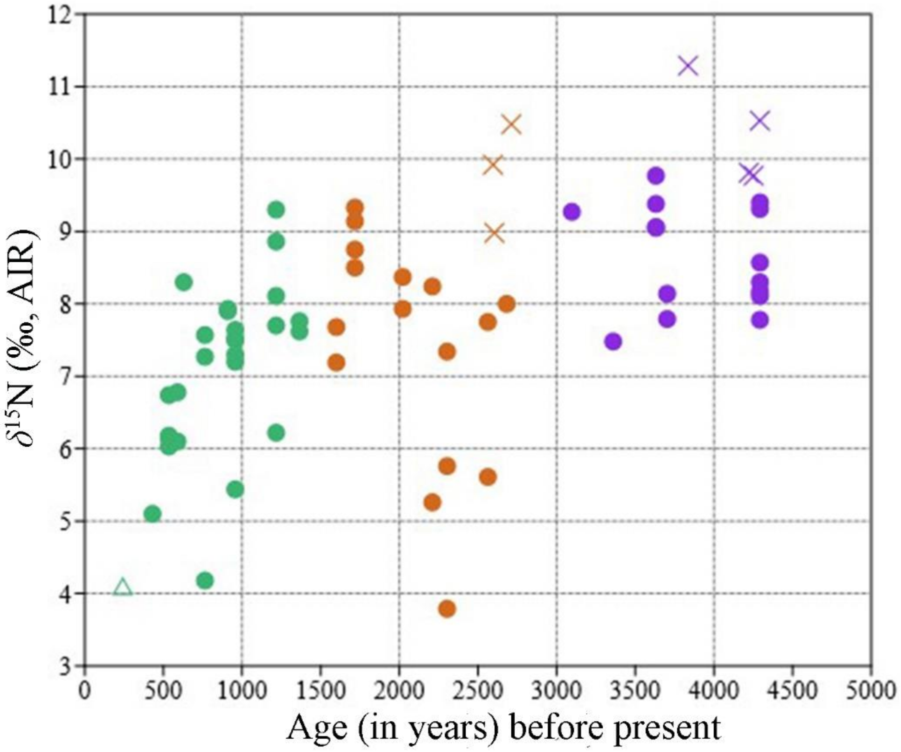

Figure 6. Scatter plot of δ^{15}N values for pigs (circles), horse (triangle), and humans (Xs) from Liang Bua. Colours indicate the temporal groups discussed in the text (Before Farming, purple; After Farming 1, orange; After Farming 2, green).

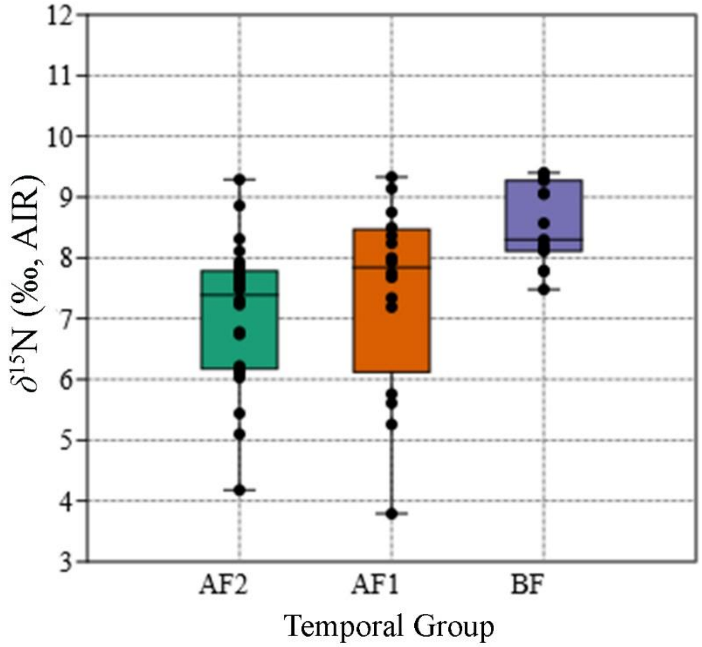

Figure 7. Box plot of δ^{15}N values for pigs from Liang Bua. Colours indicate the temporal groups discussed in the text (Before Farming, purple; After Farming 1, orange; After Farming 2, green).

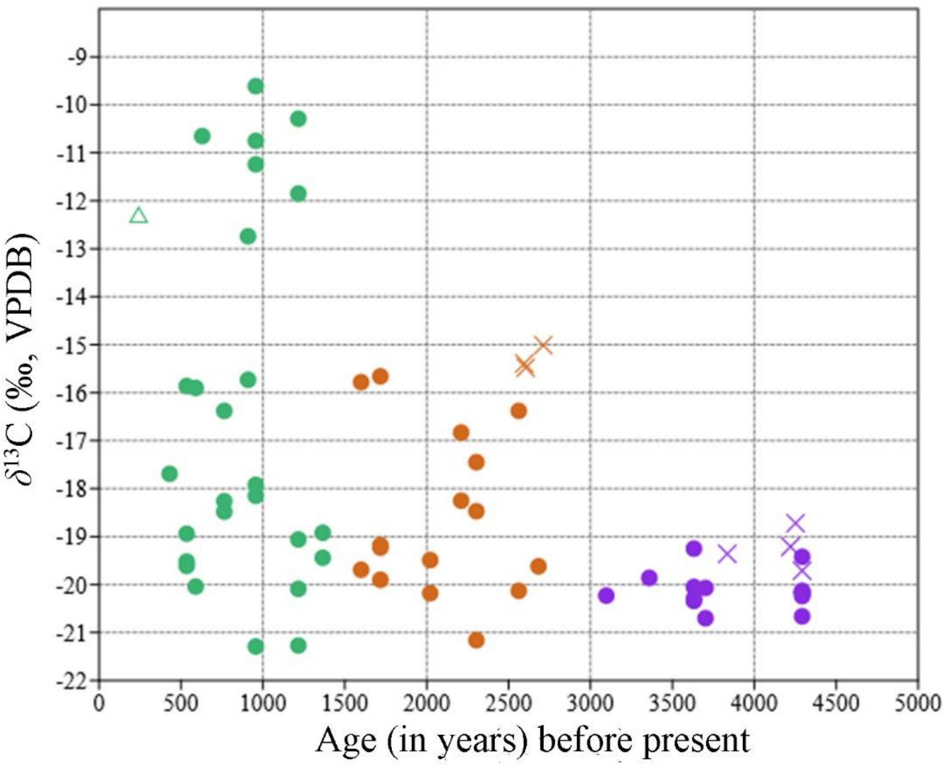

Figure 8. Scatter plot of δ^{13}C values for pigs (circles), horse (triangle), and humans (Xs) from Liang Bua. Colours indicate the temporal groups discussed in the text (Before Farming, purple; After Farming 1, orange; After Farming 2, green).

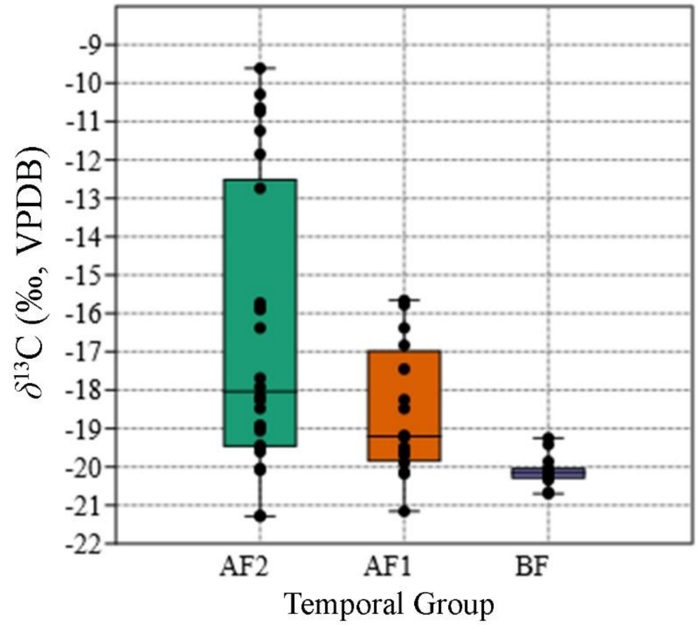

Figure 9. Box plot of δ^{13}C values for pigs from Liang Bua. Colours indicate the temporal groups discussed in the text (Before Farming, purple; After Farming 1, orange; After Farming 2, green).

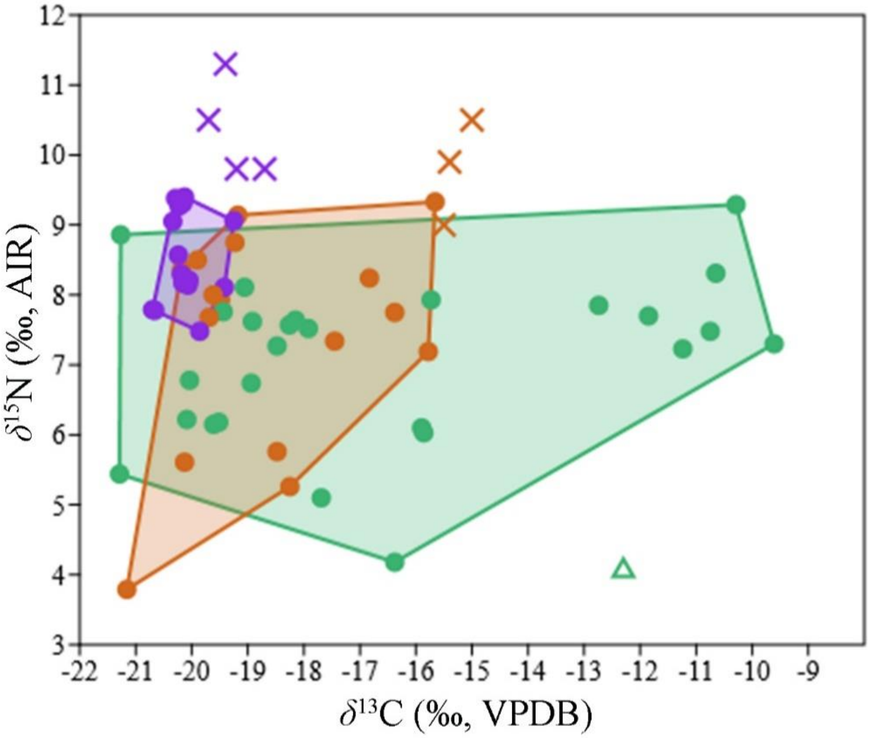

Figure 10. Comparison of the δ^{15}N and δ^{13}C values of all Liang Bua pig (circles), human (Xs), and horse (triangle) samples used in this study. Colours indicate the temporal groups discussed in the text (Before Farming, purple; After Farming 1, orange; After Farming 2, green).

Table 1. Details of the pig samples used in this thesis.

Sample ID	Sector	Modeled Age	Element
1	XXV	430	Intermediate phalanx
2	XXV	534	Lower deciduous 4th premolar
3	XXV	534	Lower deciduous 3rd premolar
4	XXV	534	Metatarsal fragment
5	XXV	534	Lower deciduous 3rd premolar
6	XXV	629	Lower deciduous 1st incisor
7	XXV	764	Rib fragment
8	XXV	764	Long bone fragment
9	XXV	764	Rib fragment
10	XXV	956	Lower 3rd molar
11	XXV	956	Cranial fragment
12	XXV	956	Cranial fragment
13	XXV	956	Cranial fragment
14	XXV	956	Cranial fragment
15	XXV	956	Upper crown fragment
16	XXV	1,216	Lower 2nd molar fragment
17	XXV	1,216	Proximal rib fragment
18	XXV	1,216	Rib shaft fragment
19	XXV	1,216	Rib fragment
20	XXV	1,216	Proximal rib fragment
21	XXV	1,717	Thoracic vertebra fragment
22	XXV	1,717	Rib fragment
23	XXV	1,717	Petrous portion of temporal bone
24	XXV	1,717	Occipital fragment
25	XXV	1,717	Upper 1st incisor
26	XXV	2,021	Upper 2nd molar
27	XXV	2,021	Long bone fragment
28	XXV	2,303	Ulna fragment
29	XXV	2,303	Cranial fragment
30	XXV	2,303	Manual proximal phalanx
31	XXV	2,562	Metapodial shaft fragment
32	XXV	2,562	Metacarpal
33	XXV	3,096	Upper 1st incisor
34	XXV	3,702	Lower deciduous 4th premolar
35	XXV	3,702	Maxillary fragment

Table 1. Details of the pig samples used in this thesis. (Cont'd)

Sample ID	Sector	Modeled Age	Element
36	XXV	4,292	Cervical vertebra
37	XXV	4,292	Calcaneus fragment
38	XXV	4,292	Calcaneus fragment
39	XXV	4,292	Maxillary fragment
40	XXV	4,292	Maxillary fragment
41	XXV	4,292	3rd metatarsal
42	XXV	4,292	Juvenile 5th metacarpal
43	XVI	589	Lower 2nd incisor
44	XVI	589	Long bone fragment
45	XVI	909	Lower 3rd molar
46	XVI	909	Phalanx
47	XVI	1,365	Lower 3rd molar
48	XVI	1,365	Lower 2nd incisor
49	XVI	1,599	Lower 2nd incisor
50	XVI	1,599	Radius fragment
51	XVI	2,210	Lower 2nd incisor
52	XVI	2,210	Intermediate phalanx
53	XVI	2,682	Lower 3rd molar
54	XVI	2,682	Metatarsal fragment
55	XVI	3,359	Calcaneus fragment
56	XVI	3,631	Molar
57	XVI	3,631	Premolar
58	XVI	3,631	Distal humerus
59	XVI	3,631	Radius fragment
60	XXV	430	Molar fragment
61	XXV	534	Tooth crown fragment
62	XXV	764	Lower incisor
63	XXV	956	Long bone fragment
64	XXV	956	Long bone fragment
65	XXV	1,216	Long bone fragment

Table 1. Details of the pig samples used in this thesis. (Cont'd)

Sample ID	Sector	Modeled Age	Element
66	XXV	1,216	Rib fragment
67	XXV	1,216	Long bone fragment
68	XXV	1,216	Long bone fragment
69	XXV	1,216	Long bone fragment
70	XXV	1,216	Scapula fragment
71	XXV	1,717	Long bone fragment
72	XXV	1,717	Long bone fragment
73	XXV	1,717	Long bone fragment
74	XXV	1,717	Long bone fragment
75	XXV	2,021	Proximal ulna fragment
76	XXV	2,021	Ulna fragment
77	XXV	2,159	Long bone fragment
78	XXV	2,159	Proximal metacarpal fragment
79	XXV	2,159	3rd phalanx
80	XXV	2,303	Cranial fragment
81	XXV	2,303	Proximal metacarpal fragment
82	XXV	2,303	Rib fragment
83	XXV	3,096	Lower 3rd molar
84	XXV	3,702	Lower incisor
85	XXV	3,702	Lower incisor
86	XXV	3,702	Lower deciduous 4th premolar
87	XXV	3,702	deciduous 2nd premolar
88	XXV	3,702	deciduous 3rd premolar
89	XXV	3,702	deciduous 4th premolar
90	XXV	4,292	Calcaneus fragment
91	XXV	4,292	2nd premolar
92	XXV	4,292	3rd premolar
93	XXV	4,292	4th premolar
94	XXV	4,292	1st premolar
95	XXV	4,292	2nd premolar
96	XXV	4,292	3rd premolar
97	XXV	4,292	4th premolar

Table 2. Details of the human and horse samples used in this thesis.

Sample ID	Sector	Age	Element
98	XXV	4,292	lower 2nd molar
99	XI	4,252	manual distal phalanx
100	XXIX	4,221	manual proximal phalanx
101	XXXII	3,835	upper premolar
102	XXXIV	2,712	pedal proximal phalanx
103	XXXIII	2,604	pedal distal phalanx
104	XXXVI	2,596	manual phalanx
105 (horse)	XXII	241	patella

Table 3. The isotopic composition of collagen for the pig samples discussed in this thesis.

Sample ID*	% Collagen	wt%C	wt%N	C:N (atomic)	δ^{15}N (‰, AIR)	δ^{13}C (‰, VPDB)	Modeled Age	Group
36	1.9	20.7	7.2	3.38	+8.3	–20.2	4,292	BF
37	1.5	30.1	10.7	3.29	+8.2	–20.2	4,292	BF
38	1.4	27.9	9.7	3.35	+8.6	–20.2	4,292	BF
39	1.5	14.7	5.2	3.32	+9.4	–20.1	4,292	BF
40	1.3	18.2	6.5	3.27	+8.1	–19.4	4,292	BF
41A**	1.6	9.0	2.9	3.68	+7.4	–20.8	4,292	BF
41B	1.6	7.9	2.6	3.59	+7.8	–20.7	4,292	BF
42	5.3	21.1	7.3	3.38	+9.3	–20.2	4,292	BF
34	1.3	33.4	11.9	3.27	+8.1	–20.1	3,702	BF
35	1.6	26.1	9.2	3.30	+7.8	–20.7	3,702	BF
56	4.8	36.5	12.8	3.33	+9.4	–20.3	3,631	BF
57A 57B	1.1	35.8	12.8	3.30	+9.0	–20.3	3,631	BF
58	1.6	36.4	12.6	3.38	+9.1	–19.3	3,631	BF
59	3.8	30.6	10.8	3.31	+8.2	–20.1	3,631	BF
55	2.3	35.9	12.7	3.30	+7.5	–19.9	3,359	BF
33	3.1	35.8	12.4	3.38	+9.3	–20.2	3,096	BF
53**	3.7	18.4	4.0	4.75	+8.1	–20.9	2,682	AF1
54	8.3	31.1	10.9	3.32	+8.0	–19.6	2,682	AF1
31A 31B	5.0	33.2	11.8	3.29	+5.6	–20.1	2,562	AF1
32	1.3	29.3	10.0	3.40	+7.7	–16.4	2,562	AF1
28	2.3	27.0	9.5	3.31	+7.3	–17.5	2,303	AF1
29A 29B	8.6	17.0	10.6	3.18	+3.8	–21.2	2,303	AF1
30	1.4	29.8	10.4	3.35	+5.8	–18.5	2,303	AF1
51	6.4	43.8	15.5	3.30	+8.2	–16.8	2,210	AF1
52	4.7	42.4	15.4	3.21	+5.3	–18.3	2,210	AF1
26	3.2	38.5	13.4	3.35	+8.4	–20.2	2,021	AF1
27	2.9	29.8	10.5	3.30	+7.9	–19.5	2,021	AF1
21	1.4	32.3	11.3	3.33	+9.1	–19.2	1,717	AF1
22**	1.2	7.8	2.2	4.09	+7.8	–20.1	1,717	AF1
23A 23B	3.8	27.1	9.4	3.39	+9.3	–15.7	1,717	AF1
24	6.8	31.6	11.1	3.33	+8.7	–19.2	1,717	AF1

Table 3. The isotopic composition of collagen for the pig samples discussed in this thesis. (Cont'd)

Sample ID*	% Collagen	wt%C	wt%N	C:N (atomic)	δ^{15}N (‰, AIR)	δ^{13}C (‰, VPDB)	Modeled Age	Group
25	4.3	39.5	13.8	3.33	+8.5	–19.9	1,717	AF1
49	14.5	32.9	10.6	3.60	+7.7	–19.7	1,599	AF1
50	2.9	33.2	11.8	3.28	+7.2	–15.8	1,599	AF1
47A 47B	5.2	36.5	12.9	3.29	+7.8	–19.4	1,365	AF2
48	15.4	38.5	13.3	3.37	+7.6	–18.9	1,365	AF2
16A 16B	5.5	40.4	13.9	3.39	+7.7	–11.8	1,216	AF2
17	10.7	35.9	12.8	3.26	+8.9	–21.3	1,216	AF2
18	19.4	41.0	14.7	3.26	+6.2	–20.1	1,216	AF2
19	13.1	34.0	12.0	3.30	+8.1	–19.1	1,216	AF2
20	6.8	38.1	13.4	3.31	+9.3	–10.3	1,216	AF2
10	4.2	41.6	14.4	3.36	+7.5	–10.8	956	AF2
11	17.9	38.8	13.9	3.25	+7.3	–9.6	956	AF2
12	18.0	43.8	15.7	3.25	+7.5	–17.9	956	AF2
13A 13B	18.3	41.6	14.9	3.27	+7.6	–18.1	956	AF2
14	18.0	36.7	13.1	3.27	+5.4	–21.3	956	AF2
15	2.6	39.6	13.8	3.35	+7.2	–11.2	956	AF2
45	7.2	42.5	15.1	3.29	+7.9	–15.7	909	AF2
46A 46B	11.7	43.9	15.7	3.26	+7.9	–12.7	909	AF2
7	13.1	42.4	15.3	3.24	+7.3	–18.5	764	AF2
8	11.7	40.6	14.6	3.25	+4.2	–16.4	764	AF2
9	15.3	43.0	15.3	3.28	+7.6	–18.3	764	AF2
6	6.0	41.5	14.7	3.30	+8.3	–10.7	629	AF2
43	9.5	44.9	16.1	3.26	+6.8	–20.0	589	AF2
44A 44B	14.3	45.4	16.2	3.26	+6.1	–15.9	589	AF2
2	8.4	42.1	15.1	3.26	+6.7	–18.9	534	AF2
3	4.7	40.2	14.1	3.32	+6.0	–15.9	534	AF2
4	6.6	42.3	14.8	3.33	+6.2	–19.5	534	AF2
5	19.5	42.9	15.3	3.27	+6.2	–19.6	534	AF2
1	20.8	44.0	15.8	3.25	+5.1	–17.7	430	AF2

*Samples with A and B are duplicates of the same sample with values shown as averages.

**Samples were excluded from further consideration within this thesis because of poor quality control indicators (C:N, wt%C, wt%N).

Table 4. The isotopic composition of collagen for the human and horse samples used in this thesis.

Sample ID*	% Collagen	C:N (atomic)	δ^{15}N (‰, AIR)	δ^{13}C (‰, VPDB)	Age	Group
98A 98B	4.5	3.46	+10.5	–19.7	4,292	BF
99	1.9	3.39	+9.8	–18.7	4,252	BF
100	2.7	3.31	+9.8	–19.2	4,221	BF
101A 101B	5.3	3.27	+11.3	–19.4	3,835	BF
102A 102B	10.0	3.23	+10.5	–15	2,712	AF1
103A 103B	2.8	3.34	+9	–15.5	2,604	AF1
104A 104B	8.2	3.22	+9.9	–15.4	2,596	AF1
105A (horse) 105B (horse)	4.7	3.45	+4.1	–12.3	241	AF2

*Samples with A and B are duplicates of the same sample with values shown as averages.

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