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Large variation in nitrogen isotopic composition of a fertilized legume

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ABSTRACT

Plant nitrogen isotopic compositions are highly variable and are influenced by a diversity of environmental and anthropogenic factors, including the application of animal-derived fertilizers. Legumes that acquire most of their nitrogen from atmospheric N_2 (rather than mineralized soil nitrogen) tend to have relatively low δ^{15}N values (consistently around 0‰), and it has been presumed that their δ^{15}N values are largely or wholly unaffected by fertilization. This study presents nitrogen isotopic data from leguminous (garden bean, *Phaseolus vulgaris*) and non-leguminous (summer squash, *Cucurbita pepo*) plants subjected to seabird guano fertilization while growing under controlled conditions. Both bean and squash tissue δ^{15}N values were substantially increased by seabird guano fertilization: +16.3 to +19.2‰ for bean and +19.6 to +24.5‰ for squash. The results of this study demonstrate that the enrichment in plant ^{15}N resulting from seabird guano fertilization occurs consistently in non-maize species. Moreover, it demonstrates that under conditions of high soil nitrogen availability, leguminous plants may obtain a substantial portion of their nitrogen through the uptake of inorganic soil nitrogen (ammonium and nitrate), rather than atmospheric N_2. In general, where the δ^{15}N values of fertilizers differ substantially from that of endogenous soil nitrogen and mineralized nitrogen derived from the fertilizer is readily available, a significant manuring effect can be expected in leguminous plants.

1. Introduction

Plant nitrogen isotopic compositions are influenced by a variety of environmental factors at global, continental, regional, and local scales. These include temperature, water availability, mycorrhizal associations, and relative openness of the nitrogen cycle (Craine et al., 2009; Handley et al., 1999; Szpak et al., 2013). Plant δ^{15}N values are strongly influenced by the application of fertilizers derived from animal waste (Bogaard et al., 2007; Choi et al., 2002; Fraser et al., 2011; Szpak et al., 2012a). Additionally, irrespective of anthropogenic influence, plants that have symbiotic associations with N_2-fixing bacteria (rhizobia) such as beans, peas, lentils, chickpeas, and peanuts (legumes, family Fabaceae) consistently have δ^{15}N values around 0‰ (Fig. 1). Legume δ^{15}N values are typically lower than non N_2-fixing plants (Yoneyama et al., 1986) because they derive a portion of their nitrogen from atmospheric N_2, which has a nitrogen isotopic composition of 0‰ (Mariotti, 1983). There is little fractionation of ^{15}N during N_2-fixation (Kohl and Shearer, 1980), resulting in plant tissues with nitrogen isotopic compositions consistently around 0‰.

Legumes have been significant components of prehistoric agricultural systems throughout much of the world, including: Europe (Bogaard, 2004), the near east (Abbo et al., 2009), Africa (D'Andrea et al., 1999), South Asia (Fuller and Harvey, 2006), East Asia (Crawford et al., 2005), North America (Hart et al., 2002), Mesoamerica (Staller and Carrasco, 2010), and South America (Pearsall, 2008). Because of their high protein content relative to other plants, legumes can represent a substantial portion of protein in human diets (Graham and Vance, 2003). The proteinaceous component of the diet is that which is most commonly assessed in isotopic paleodietary studies because the tissue most frequently analyzed (bone collagen) is derived principally from dietary protein (Jim et al., 2004). It is therefore especially crucial that the range and variation in carbon and nitrogen isotopic compositions of leguminous plants be understood. Regional surveys of cultivated plants provide one means towards this end (Szpak et al., 2013; Warinner et al., 2013), but because the nitrogen isotopic compositions of any plants collected from modern contexts are strongly influenced by local agricultural practices (which are unlikely to be analogous with those practiced in antiquity), these data must be interpreted cautiously in paleodietary analyses. Studies of controlled settings

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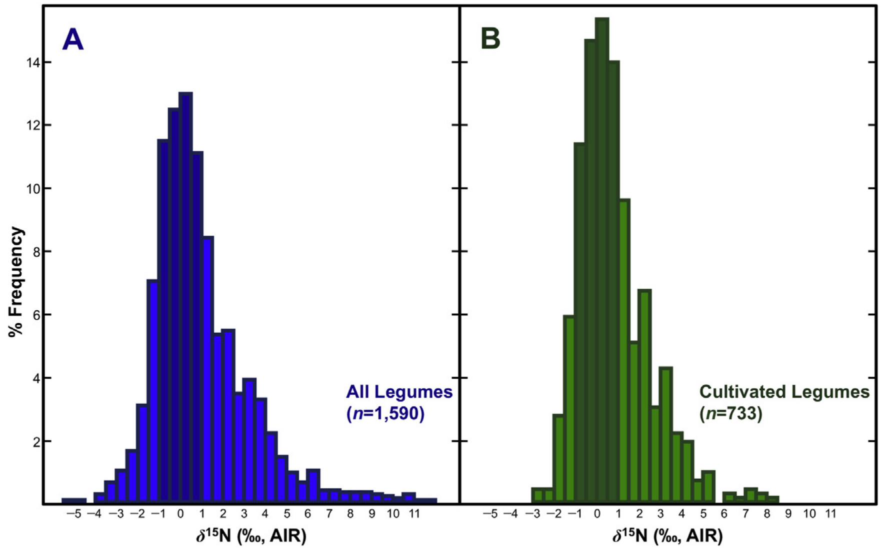

Fig. 1. Histograms representing nitrogen isotopic compositions of leguminous plants. Data were taken from published literature (see Appendix) and are for above-ground plant tissues only. (A) Measurements from both cultivated (e.g. bean, pea, chickpea, groundnut) and wild (e.g. clover, *Acacia* spp., *Inga* spp.) species. (B) Measurements from cultivated, edible legumes only. The darker shaded bars represent $\delta^{15}N$ values between −1 and +1‰, which represent 47.8% of the surveyed measurements for all legumes and 55.1% of the cultivated legumes.

are necessary to fully understand the complexities of plant nitrogen isotopic compositions.

It has been suggested that leguminous (N_2-fixing) plants are minimally affected by manuring relative to non-legumes (Bogaard et al., 2013; Fraser et al., 2011). On the basis of nitrogen isotopic data from a number of long-term experimental fields, Fraser et al. (2011) concluded that legume $\delta^{15}N$ values are not affected, or minimally affected, by manure application unless the application is at a very high rate over the long term. Aside from the rate and duration of application, an important consideration in terms of the effects of manuring on plant $\delta^{15}N$ values is the *type* of manure used. While in the vast majority of cases, nitrogen isotopic compositions of manured plants tend to be higher than unfertilized plants, the magnitude of this difference is highly variable, both between and within different fertilizer types.

The purpose of the present study was to examine the effects of seabird guano on the nitrogen isotopic composition of a nitrogen-fixing plant (common garden bean, *Phaseolus vulgaris*) in a controlled setting. Seabird guano is a fertilizer that was of tremendous agricultural importance in the nineteenth century and was mined extensively from nearshore islands off the western coast of South America from the 1840s through to the 1870s (Cushman, 2013). This fertilizer may also have been used in by various groups in the Andes prior to the arrival of Europeans (Garcilaso de la Vega, 1966; Julien, 1985; Kubler, 1948; Netherly, 1977; Nordt et al., 2004). In addition to having implications for dietary reconstruction in the Andean region of South America (see Szpak et al., 2012a), the high nitrogen isotopic composition of seabird guano ($\delta^{15}N > +20‰$) makes assessment of the relative importance of fertilizer- and atmospheric-nitrogen to plant growth much less ambiguous than other fertilizers (e.g. cattle or sheep manure).

2. Materials and methods

2.1. Experimental design

Common beans (*Phaseolus vulgaris* L., garden bean 'provider', The Cook's Garden®, Lot 3, 2010) and summer squash (*Cucurbita pepo*, early summer crookneck, Burpee®, Lot 6, 2010) were grown in a walk-in growth chamber at the Biotron Institute for Experimental Climate Change Research at the University of Western Ontario. Growth chamber conditions were: 25/18 °C (day/night temperature), 13 h photoperiod (185 W fluorescent bulbs), 60% relative humidity (Szpak et al., 2012b). Both beans and squash (four replicates each) were grown in 2 L free-draining (perforated at the base) plastic containers; substrate was Pro-mix® for containers (75–85% sphagnum moss, 15–25% perlite and limestone).

Seedlings were sprouted in the 2 L containers in the absence of any fertilizers. Peruvian seabird guano (Guano Company International) was applied to the surface of the soil at a rate of 5 g/container 5 days after seedling emergence (four replicates for beans and squash). The nitrogen isotopic composition of the guano was previously determined to be +26.7 ± 0.6‰ (Szpak et al., 2012b). Four replicate controls (no fertilizer applied) of both beans and squash were grown simultaneously under the same conditions. Leaf and fruit samples were harvested 65 d after planting.

2.2. Sample preparation

Leaf and fruit samples were cleaned of visible foreign matter and frozen (−25 °C) immediately after sampling. Leaf samples consisted of entire leaves excluding petioles; for squash three leaves were sampled and for beans all leaves on each plant were sampled. Fruit samples consisted of entire fruits for squash. For bean fruit samples, all beans within the largest pod at the time of harvest were homogenized; the pod was not included in the sample. Additionally, for one control and one fertilized plant, single beans from the second largest pod at the time of harvest were processed individually to assess any potential within-fruit variation in nitrogen isotopic composition. Prior to isotopic analysis, samples were dried at 90 °C for at least 48 h (leaves) or 96 h (fruit) and then ground to a homogenous fine powder using a mechanical shaker (Crescent Wig-L-Bug). This material was subsequently dried at 90 °C for at least 48 h.

Two commercial samples of Peruvian seabird guano were also analyzed for comparative purposes: Original Sea Bird Guano (Guano Company International, reported N–P–K 13–12–2) and Peruvian Seabird Guano (Sunleaves Garden Products, reported N–P–K 12–11–2). One ∼50 g sample was taken from each of five