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Investigating Local Plant Food Systems Through

Stable Isotope Analyses

by

Katherine C. Roberts

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Investigating Local Plant Food Systems Through Stable Isotope Analyses

Thesis Abstract- Idaho State University (2019)

This thesis investigated where and how Pocatello, Idaho’s leafy green retail foods (romaine lettuce, spinach and kale) are cultivated compared to another intermountain west city with similar demographics (Missoula, MT). \( \delta^{18}O \), \( \delta^{13}C \) and \( \delta^{15}N \) stable isotope analyses were used to characterize edible greens in each city. Collectively, the data supported the hypothesis that Pocatello’s retail food never becomes local, while in Missoula, MT, truly local produce was available in retail grocery stores, although only during the summer and early fall. Although \( \delta^{15}N \) did not provide absolute certainty about nitrogen cultivation methods, it can provide a framework for investigating potential nitrogen sources in plant food. Efforts to produce local leafy greens during the winter months when retail foods move to more southern locations were focused on resilience and sustainability. Utilizing winter annual plant life histories and sustainable designs allowed local leafy greens to be produced during the winter months in Pocatello.

Key Words: leafy green, retail food, stable isotope, edible greens, local, cultivation methods, resilience, sustainability, winter annual
**Introduction**

As our food system has become a global enterprise and large amounts of foods are made available throughout the world for consumption, dietary patterns have changed (e.g. decreased food diversity in diets of residents urbanized areas) (Nardoto et al. 2006). While most U.S. consumers may not realize it, our relationship with what we actually eat has simultaneously become disconnected. Most Americans have lost the historically intimate, biological knowledge previously associated with their food. For example, in the early 1900’s, nearly 50 percent of Americans lived or worked on a farm; now this demographic is down to about 2 percent (McKay 2017). This change was spurred by large machinery becoming more efficient and by farms increasing in size, as American farmers were encouraged to “get big or get out” by the U.S. Secretary of Agriculture during the 1970’s (McKay 2017). This trend towards large scale industrialized farming was further enabled by emerging food distribution networks that relied on unprecedented efficiencies in new ground and air transportation infrastructure (Olmstead and Rhode 2017). In turn, raw, unprocessed plant foods began traveling much larger distances between soil and plate. Additionally, highly processed and packaged convenience foods became a strong market force; corn became calendar year high fructose corn syrup instead of summertime corn on the cob. This transition has conditioned contemporary consumers to become accustomed to exotic foods grown at great distances from the points of consumption, and to expect their availability year-round, as “out-of-season” here has become “always-in-season”, but from somewhere else. The new food system also dismantled long-standing culturally conditioned relationships to meal planning and cooking (Pollan 2009), with family food traditions now mostly relegated to special events.
like holidays. Our detached sensibilities about the origins of our own food are reasonable considering we now have almost everything available at our fingertips and the actual points and processes of production have all but disappeared from view. While this outcome might be understandable, it also increasingly is recognized as potentially undesirable.

Although movements back toward more locally sourced regional food systems are occurring, problems still remain. In Idaho for example, much of the region’s raw produce is also available in retail supermarkets among states throughout the western U.S., and it commonly originates from farms in California and Oregon. California has experienced some of its worst droughts on record over the last six years due to global hydrological patterns and climate change (Meng et al. 2016, Gnaneswar 2017). Climate change models predict similar extreme events in the future, and changes in average climatic conditions may present challenges in stable food production (Gornall et al. 2010, Knapp and Heijden 2018).

Even in the absence of unpredictable impacts of climate change, the rapid proliferation of agribusiness as a primary means of food production and distribution has led to a heavy local reliance on a food system with many unprecedented and negative attributes, including widespread use of pesticides, synthetic fertilizers, and a lack in crop and genetic diversity (Muller et al. 2017). Large scale, high throughput methods of agriculture have increased absolute levels of food production, but these practices have also played a role in disturbing the nitrogen cycle by providing excessive fixed nitrogen into sensitive water systems (Yang and Gruber 2016). Contemporary commercial farming techniques can indeed increase agricultural yields in the short term, but only in the
context of undervalued ecological and environmental costs that ultimately represent a wide spectrum of costs to human health and wellbeing (Freedman and Bess 2011).

Perhaps acknowledging the need for change, consumer demands for local and more environmentally friendly foods have been on the rise. Ironically, this market force suggests an emerging interest in reviving features of an older model for food production and distribution. Consequently, some communities are recreating some of the prevailing practices that predated the establishment of agribusiness, allowing perishable foods to be more readily sourced to local farms and farmers. These evolving consumer attitudes help to explain the dramatic increase in the number of farmer’s markets and local foods available throughout the United States (Martinez 2010).

Conventional retail grocery suppliers have also responded by endeavoring to identify their raw plant foods as “locally grown”, presumably to impart the same impression on their consumers that farmer’s markets do. An important question emerging in this retailer trend is what constitutes “local” verses not? Interpretations of what “local” food means varies widely among grocery retailers (Dunne et al. 2011), but a general consensus is that local implies the distance between production and consumption (i.e., farm-to-fork) has been reduced as much as possible (Peters et al. 2009). During 2008, the Farm Act stated that in order to be considered local, the total distance between the point of origin and where the final product is marketed is less than 644km (400 miles; Peterson 2008). However, even this definition is not universally accepted and has since been modified due to social, geographical and supply chain characteristics (Martinez et al. 2010). In terms of geography, population density can also influence the meaning of local food. For example, areas that have lower population densities may identify particular
foods as local, even when consumers in urbanized areas would deem foods traveling an equivalent physical distance to be too far away to qualify as local (Martinez 2010). At the grocery chain level, going local may imply a shift to farm suppliers in an adjacent state rather than several states (or countries) away. At the farmer’s market level, local is more likely to imply food that can be sourced to farms in the same or adjacent counties. In this latter vernacular, the local food movement allows interested consumers to have a more personal relationship with food by investigating or even taking part in its cultivation or harvest (e.g., “pick you own” farms; CSA’s: community shared agriculture). At a minimum, the consumer is more likely to be reconnected with their farmers. For our purposes, local will be defined as 161km (100 miles) from the site of production (i.e., plants in the soil) to the point of consumption (i.e., food on the fork). The focus of the current study is on edible plants, although locally sourced animals that enter the food system are another legitimate aspect of the same problem.

As a result of interested consumers trying to become more familiar with where their food is grown, mechanisms of production also come into focus. Food systems that include small scale local farming are more likely to include more organic practices, which is farming without synthetic chemicals such as herbicides, pesticides and artificial fertilizers (Muller et al. 2017). Given the detrimental environmental effects attributable to conventional agriculture, alternative methods like organic farming have received renewed interest to determine if these environmentally friendly modes of production can meet the demands of a growing global population; over the last 20 years, land dedicated to producing organic agriculture has steadily increased (Kniss et. al 2016, Muller et al. 2017). A return to unprocessed local plant foods, with benign impacts on environments
and social benefits linked to production (e.g., stimulating the local economy), is a potentially key part of any set of integrated solutions needed to ensure a lasting, sustainable food production system. Sustainability encompasses methods of agricultural production that provide farmers with a fair income coupled with environmentally friendly production practices that benefit communities (Munasib and Jordan 2011). Initiatives to help reduce food waste and to foster shifts towards a plant-based diet can also help transition toward more sustainable practices (Ripple et al. 2017, Shepon et al. 2018). Smaller scale food systems that integrate local farming also minimize widespread dissemination of food-borne diseases. For example, the Centers for Disease Control and Prevention reported that a recent outbreak of *Escherichia coli* from Romaine lettuce appears to be over after an investigation that lasted over seven weeks with sixty-two cases of illness and twenty-five hospitalizations across seventeen states (Nutrition 2019). Reoccurring instances such as this advocate for reducing our exclusive reliance on a food system based on industrialized agriculture; local food production, including organic farming methods, helps to foster favorable ecological impacts which contribute to better individual health and supports local food-making subsystems that can be more realistically sustained for years to come (Jones et al. 2019).
Chapter 1

$\delta^{18}O$ ISOTOPE ANALYSIS OF EDIBLE LEAFY GREENS IN THE INTERMOUNTAIN WEST

Abstract

$\delta^{18}O$ and $\delta^{13}C$ stable isotope analyses were conducted to characterize a local food system in Pocatello, ID and compare a sister city with similar demographics (Missoula, MT). Patterns of geographic variation in stable isotopes of bound water (i.e., $\delta^{18}O$ and $\delta^{13}C$) were used to estimate the approximate locations of origin of three leafy green vegetables (romaine lettuce, spinach and kale). Tracking leaf dry mass $\delta^{18}O$ can potentially detect changes in sites of agricultural cultivation based on geographic and seasonal variation. Collectively, the data supported the hypothesis that Pocatello, Idaho’s retail food never becomes local (161km), while in Missoula, MT, local produce was available in retail grocery stores only during the summer and early fall. However, during the late fall, winter, and early spring, sites of production become decidedly less local in both Pocatello and Missoula when day lengths and temperatures decrease; production sites converge on locations much further south.
Introduction

A Place-based Study in Pocatello, ID

Motivating a return to local food systems is expected to rely on improving local consumer literacy about their food system, region by region. Consequently, knowing more about where retailers’ food is from and how it is produced is a vital diagnostic tool to characterize the current features of a community’s food system. Illuminating the prevailing circumstances can help determine whether the food system meets current community expectations and suggests potential strategies for any improvements deemed necessary to align with those expectations. It is also possible that simply increasing community literacy about existing food production and distribution practices will actively drive attitudes that favor useful revisions to the local food system. Of course, specific efforts to reinvent a local plant food system must be responsive to local opportunities as well as constraints; the range of agricultural possibilities for plant food production is strongly dependent on a community’s geographic location and seasonal climate. These attributes will vary, sometimes dramatically, by region. This thesis sought to develop some of the foundational knowledge needed to improve local consumer food system literacy about edible leafy greens, using Pocatello, Idaho as a representative case study.

The current project is predicated on the assumption that the average retail consumer in Pocatello endeavoring to eat a plant-based diet probably has little or no knowledge where their grocery suppliers’ fresh produce comes from. Consequently, the impacts of efforts to “go green” and eat more fresh plant produce in lieu of meats may still implicitly and unwittingly endorse many of the negative practices of modern agriculture, particularly in winter. A primary goal of the current research is to determine
where representative perishable plant foods (i.e., leafy greens) in this local market originate during the calendar year. The study investigated the feasibility of making inferences about the locations of plant crop cultivation based on analysis of $\delta^{18}O$ from bound water in dry mass of plant foods because $\delta^{18}O$ is known to vary geographically, seasonally and by elevation in North America (Fig. 1.1; Bowen and Wilkinson 2002). Thus, plant foods might carry a characteristic $\delta^{18}O$ signature suggesting their locations of origin. The ultimate motivation is to better educate interested food consumers in the Pocatello area about one aspect of the food system they commonly participate in and rely on. The working hypothesis is that most perishable plant foods are “snow birding” in Pocatello’s winter retail food market (i.e., sites of production move to more southern latitudes in winter), so they are decidedly not local. Although sites of plant food production are expected to get closer to Pocatello during the summer months, a second related hypothesis is that summer sources of plant produce available in supermarkets never “migrate” far enough to become truly local (\(\leq 161\) km i.e., 100 miles, in Pocatello). The apparent availability of “local” plant food in the summer is based on retail grocers’ liberal and flexible characterization of what “local food” means in relative terms.

**A Comparable Intermountain West City: Missoula, MT**

The existence of centralized plant food production and distribution systems dominated by a relatively small number of agribusiness interests suggests many communities in geographically separate locations still have identical retail supply chains. In effect, people living in different cities are consuming plants supplied from the same large-scale growers and eating the same food. To interrogate this idea, plant food
availability in a sister city in the Intermountain West with comparable population and climatic characteristics was integrated into the study. Missoula, MT is ~4° N latitude of Pocatello, so any opportunities and constraints for the development of a local plant food system are expected to be similar between cities, a priori. If regional food systems are truly the product of local geographical climatic constraints, Pocatello and Missoula are expected to converge on food systems with the same basic supply features. Alternatively, any notable differences between cities might highlight untapped opportunities for improvements in the local food system in either city.

**Materials and Methods**

**Study Sites**

_Pocatello, Idaho_ is a located in the intermountain west with a total population of 54,228 (Demographics- City of Pocatello 2018). Pocatello’s Portneuf Valley sits ~1363 meters in elevation and is located at 42.87° N, -112.45° W, with 2018 mean minimum annual temperatures of 1.56° and annual mean maximum temperatures of 16.56° C, 2017 annual snowfall of 179.3 cm and total liquid content of 16 cm (Climate Data Online- National Climatic Data Center 2019).

Criteria are available to objectively classify a retailer as a supermarket, including four or more cash registers, twenty or more fruits and vegetables available, and fresh milk, meat, and produce sections (Han et al. 2012). By these metrics, the Pocatello area has at least nine food stores that qualify as supermarkets. Additionally, one local farmer’s market, the Portneuf Valley Farmer’s Market, runs seasonally from the first Saturday in May until the last Saturday of October (Old Town Pocatello Markets 2018). One organic
grower also currently sells directly to consumers from a roadside farm stand on Tuesdays from July until mid-November. Thus, in Pocatello’s cold temperate desert climate, the conventional growing season allows motivated consumers some access to local plant foods, but mainly during late spring, summer and early fall months, and only outside the mainstream commercial grocery retailer setting.

**Missoula, MT** is located in the intermountain west with a total population of 72,072 (Missoula, Montana Population 2018 (Demographics, Maps, Graphs) 2018). Missoula sits ~978 meters in elevation and is located at 46.87° N, -113.99° W, with annual mean minimum average annual temperatures of 1.1° and annual maximum temperatures of 13.9° C in 2018 (Climate Data Online - National Climatic Data Center 2019). During 2017, Missoula had a total liquid content of 38.7 cm and annual snowfall of 166.6 cm (Climate Data Online - National Climatic Data Center 2019).

Missoula has at least 10 food stores that have criteria that classify them as supermarkets, at least two specialty stores (stores that sell specific products exclusively i.e. fruit/vegetable stores, bakery, etc.) (Han et al. 2012) and two local farmer’s markets. Both the Missoula Farmers’ Market and Clark Fork Market run from the first Saturday in May until the last Saturday in October (Missoula Farmers Market 2018; Clark Fork Market - Saturday Farmers’ Market - Missoula, Montana 2019). Thus, Missoula offers food at farmer’s markets in essentially the same seasonal time frame as Pocatello. Additionally, one retail grocery store claims to have locally grown produce available in season (i.e., plant foods grown within a 161km radius of the city). This retailer was
specifically integrated into the study because it seemed to rely on a unique plant food system supply model while still meeting the criteria as a retail supermarket.

**δ¹⁸O Analysis of Plant Foods**

*Verified samples.* A pilot study was conducted to determine whether oxygen isotope composition in bound water of plant foods varied reliably by the latitude of cultivation in North America, parallel to observed geographic variation in surface precipitation (Fig. 1.1). Samples of red potatoes from Alaska, Idaho, Nevada, Montana, and California, and radishes from Arizona, were obtained in October 2017 from geographically dispersed local gardens or farmers’ markets. The distribution of samples relied on opportunistic collections made during travel by study collaborators and shipments from geographically dispersed personal contacts. Farmer’s market vendor interviews were used to improve confidence about farm location and farm water sources. These samples were deemed “verified” i.e. highly likely to have grown in a specific location with a regionally local water source. Water isotope composition varies seasonally as well as geographically (Feng et al. 2009). Consequently, food samples included in the study were coded as “summer” and “winter” based on when the most likely period of cultivation fell on the calendar (e.g., potato samples harvested in early fall were a “summer” crop; spinach leaves purchased in March were a “winter” crop).

Stable isotope analysis of oxygen was performed on five sub-replicate samples taken from each location excluding Nevada, where two sub-replicate samples were initially analyzed. Samples were oven dried at 50-70°C for at least 48 hours, and hand ground by a mortar and pestle that was previously rinsed with distilled water and let dry
for at least 24 hours. Samples were analyzed using a ThermoFinnigan High Temperature Conversion Elemental analyzer (TC/EA) interfaced to a Delta V Advantage isotope ratio mass spectrometer through the ConFlo IV system. The samples were weighed in silver capsules and loaded into a Zero-Blank Autosampler (Costech Analytical, Valencia, CA, USA). Samples were combusted at 1400°C in a glassy carbon reactor, and resultant gases were separated in a GC column at 85°C and subsequently fed into the isotope ratio mass spectrometer for analysis. Estimates of $\delta^{18}O$ are reported as ‰ values relative to the VSMOW scale. International/certified standards were analyzed concurrent with the samples to normalize the raw data and monitor accuracy. Precision was estimated to be \( \leq 0.2 \) ‰ for $\delta^{18}O$.

During the course of the study period, thirteen additional verified samples collected during the calendar year were added, including some from plants grown outdoors under glass during the winter in Pocatello, ID and Missoula, MT (Fig. 1.2). The analysis was expanded to include longitude as a geographic variable because it appeared retailer’s plant foods were not sourcing to locations along a strictly north-south longitudinal axis (see unverified samples, below). Three more of the Nevada study sub-samples were analyzed from stored tissue dry mass (making each of the pilot study locations have a total of five sub-replicate samples) using the method of $\delta^{18}O$ analysis described above, after re-drying each sample overnight at 60°C.

**Unverified samples.** Unverified plant food samples were those purchased from the commercial retail grocery supply chain. Some store samples were labeled with specific farm or distributor location data and others were either geographically vague or lacking
any specific point of origin data. When available, a record of location of origin from product labels was recorded. For this facet of the work, three twist-tied bunches of retail spinach leaves, three heads of green leaf lettuce/romaine lettuce, and three twist-tied bunches of kale from two Pocatello grocery stores (ID1 and ID2) were purchased at two times of year. The winter season purchase was on March 18, 2018 and the summer season purchase was on June, 15, 2018.

Samples from Idaho were rinsed with distilled water, patted dry, and stored in sealed plastic bags at ~ -8 °C until they were processed for analysis. Montana summer samples of chard, cauliflower, bell pepper, and purple kale were obtained from a grocery store in Missoula, MT (MT1) on September 16, 2018. Additionally, Montana winter samples of three individual spinach leaves (in this instance, the spinach leaves were selected from a “mixed salad greens” display sourced to “Mexico and USA”), three heads of romaine lettuce, and three twist-tied bunches of kale were obtained on January 13, 2019. Samples from Missoula were transported in a cooler to Pocatello and oven dried (without frozen storage) until further processing. Immediately before final processing, all unverified samples were oven dried at 50-70° C then hand-ground by mortar and pestle. Aliquots of tissue dry mass (0.3-0.35 mg) were weighed after re-drying each sample overnight at 60°C. These unverified samples were analyzed for δ¹⁸O as described above for verified samples.

δ¹³C Analysis. In ideal agricultural settings, precipitation supplemented by irrigation water will reduce or eliminate crop plant water stress even while allowing high rates of leaf evapotranspiration. Since evaporative water loss from leaves can also influence δ¹⁸O
and the unverified samples in this study specifically focused on edible leaves, estimates of δ\textsuperscript{13}C were also compiled as a means to broadly assess levels of crop leaf water stress. Stomata, which control the exchange of CO\textsubscript{2} and water, expand and contract to maintain adequate levels of CO\textsubscript{2} within plants while simultaneously preventing water loss (Lawson and Blatt 2014). The physical diffusion of CO\textsubscript{2} into leaves and photosynthesis can both result in carbon isotope fractionation, and both processes discriminate against the heavier isotope (\textsuperscript{13}C) (Dawson et al. 2002). Consequently, δ\textsuperscript{13}C is often used to estimate plant productivity and the level of water availability (Zhang et al. 2018). In general, plants that are more water stressed will have more enriched (i.e., positive) δ\textsuperscript{13}C values (Lawson and Blatt 2014).

Verified and unverified samples used in the δ\textsuperscript{13}C analysis were used to investigate if sampled crops experienced water stress. Verified pilot study potato samples and unverified winter Montana were not included in the δ\textsuperscript{13}C analysis due to δ\textsuperscript{13}C study occurring prior to collected winter Montana samples. Aliquots of tissue dry mass (2.5 mg) were weighed into 4mm x 6mm tins. δ\textsuperscript{13}C stable isotope analysis was performed on three sub-replicate samples taken from each verified location. For unverified samples, three sub-replicate isotope samples were taken from one randomly selected spinach, lettuce and kale sample from each both ID1 and ID2.

Estimates of carbon elemental concentrations as a percent of total dry mass and stable isotope ratios (\textsuperscript{13}C/\textsuperscript{12}C) were done by the Idaho State University Interdisciplinary Laboratory for Elemental and Isotopic Analysis (ILEIA) using a Costech ECS 4010 elemental analyzer interfaced to a Thermo Delta V Advantage continuous flow isotope ratio mass spectrometer. Stable isotopic data are reported in standard delta notation.
(\(\delta^{13}\)C) relative to the Vienna PeeDee Belemnite (VPDB) reference standards. Analytical precision, calculated from analysis of standards distributed throughout each run, was \(\leq \pm 0.2\%\) for both carbon stable isotopes, and \(< \pm 0.5\%\) of the sample value for %C.

**Statistical Analysis**

*Verified samples.* Estimates of verified plant food \(\delta^{18}\)O in the pilot study were analyzed by linear regression to determine if a significant negative correlation existed between latitude of cultivation and \(\delta^{18}\)O. Subsequently, the full set of verified samples was analyzed using a multiple regression model with the lowest AIC (56.529, to avoid an overfit model) and was analyzed with an ANOVA. The model with the lowest AIC showed latitude, longitude, season, elevation, and vegetable type all influence \(\delta^{18}\)O.

*Unverified samples.* Location of origin for unverified samples from in-store labels could not be independently corroborated, so the analytical focus was on \(\delta^{18}\)O irrespective of presumptive longitude and latitude of food origin. A type III analysis of variance (ANOVA) was used to account for unbalanced sample sizes with Satterthwaite’s method for denominator degrees of freedom to account for a mixed effect model. Due to unequal sample sizes, a Mann-Whitney one-tailed t-test was used to compare pooled summer and winter means from all stores. Unpaired, one tailed t-tests were used to compare summer means and winter means by season and store for ID1 and ID2. MT1 had unequal sample sizes; therefore, a Mann-Whitney one-tailed t-test was used to compare MT1 summer and winter means. Statistical analyses were performed using the R statistical program (R Core Team 2014) with reference to *Foundational and Applied Statistics for Biologists using R*
T-tests were performed using GraphPad Prism version 8.0.2 (GraphPad Prism version 8.0.2 for Mac OS X 2019).

**Results**

**Verified Samples.** Linear regression analysis of pilot study potato samples revealed a significant negative relationship between $\delta^{18}O$ and latitude of cultivation ($R^2 = 0.79$, $p = 0.017$) (Fig. 1.3, Table 1.1). Multiple regression analysis and ANOVA of all verified samples further revealed a significant relationship between main effects of latitude, longitude, season, and vegetable type ($R^2 = 0.8795$, p-value: $6.538 \times 10^{-16}$) (Fig. 1.4, Table 1.1 and 1.2). Plant food type (i.e., “vegetation”) and latitude were implicated as significant effects in the model with strong statistical confidence (p-values: $1.75 \times 10^{-7}$ and $2.001 \times 10^{-7}$ respectively); verified crops included eight different varieties of vegetables (Fig.1.5).

**Unverified Samples.** Analysis of variance for unverified samples indicated a significant interaction between store and season on $\delta^{18}O$ (p-value: 0.0039) (Tables 1.3 and 1.4). Patterns of variation in $\delta^{18}O$ by seasons among individual stores were not uniform (Fig. 1.6; Table 1.3). ID1 leafy greens exhibited lower average $\delta^{18}O$ during the summer verses the winter (p-value: 0.0002). ID2 produce did not exhibit significant differences in mean $\delta^{18}O$ by season (p-value: 0.3527). MT1 summer leafy greens had lower $\delta^{18}O$ during the summer verses the winter (p-value: 0.0114).

Although there was no mechanism to independently verify the accuracy of store labels indicating location of crop origin, there was no reason to expect this consumer
information was incorrect or intentionally misleading. Under the assumption that available location data provided by retailers for unverified foods (Table 1.3) were accurate, it was possible to evaluate the apparent change for several crop locations from winter to summer in ID1, ID2 and MT1 in relation to corresponding changes in dry mass $\delta^{18}O$. To estimate geographic changes in crop location from store label data, the latitude (and longitude) of a crop’s summer location at one retail grocery was subtracted from the latitude (and longitude, respectively) for the same crop’s winter location at the same store. To estimate the seasonal change in a crop’s $\delta^{18}O$ at given grocery, the seasonal difference between $\delta^{18}O$ (e.g., ID1 mean $\delta^{18}O$ of lettuce in winter – ID1 mean $\delta^{18}O$ of lettuce in summer) was calculated (Table 1.5). This analysis suggested at least two potential patterns. Some crops remained in nearly the same geographic locations according to store label information (e.g., ID1 lettuce) while $\delta^{18}O$ shifted seasonally by nearly 9‰. Alternatively, some crops apparently moved significant geographic distances with no apparent change in $\delta^{18}O$ from summer to winter (e.g., ID1 spinach; MT1 kale, Table 1.5).

Estimates of leaf dry mass $\delta^{13}C$ in verified samples were between -30‰ to -26.3‰. Beet leaves from Missoula, MT had the highest $\delta^{13}C$, while kale sampled from Logan, UT had the lowest $\delta^{13}C$ (Table 1.6). Unverified samples exhibited a similar range in $\delta^{13}C$, from -30.1‰ to -25.9‰ (Table 1.7).

**Discussion**

At the outset of this research, the geographic locations of edible leafy greens in Pocatello, ID were hypothesized to be “snow birding” from northern latitudes in summer
to more southern latitudes in winter, and this seasonal movement was expected to be
evident as changes in leaf dry mass $\delta^{18}$O sampled from the city’s retail grocers. Using
$\delta^{18}$O to evaluate the main study hypothesis proved to have potential (Figs. 1.1, 1.3, 1.6;
Table 1.2), but the confounding effects of seasonal fluctuation in $\delta^{18}$O (Table 1.4)
combined with potential changes in geographic location added an unexpected level of
complexity to interpretation of the patterns of variation in this particular group of plant
foods. Many agricultural fields are watered with either or both groundwater and
precipitation (Maupin 2018, Evaristo and McDonnell 2017), so $\delta^{18}$O in any particular
plant food dry mass sample can integrate a combination of water sources. Groundwater
$\delta^{18}$O typically remains fairly resistant to seasonal fluctuations and is consistent with
annually weighted averaged values of surface precipitation $\delta^{18}$O (Maupin 2018). This fact
argues for limited seasonal variation in plant food $\delta^{18}$O if crops are primarily irrigated
with geographically local ground water. While seasonal variation in groundwater $\delta^{18}$O is
typically low, variation in surface precipitation is influenced by latitude, longitude, and
temperature driven variation (i.e. season and elevation) (Fujimoto et al. 2014, Bowen et
al. 2005). Consequently, coastal regions with moderate climates typically have small but
significant seasonal fluctuations in precipitation $\delta^{18}$O compared to regions further inland
(Fig. 1.7; Feng et al. 2009).

If leafy greens in agriculture are prone to directly incorporate local surface
precipitation and (or) be under the influence of physical processes that fractionate water
isotopes (e.g., evaporation from soil surfaces near the root zone; leaf evapotranspiration;
Barbour, 2007) in ways that depend on seasonality, tissue dry mass will ultimately
integrate variation due to both season of cultivation and geographic location. This
possibility drives expectations about variation in $\delta^{18}O$ in the current study in important ways. Geographic variation in $\delta^{18}O$ alone (Fig. 1.1) predicts foods irrigated with ground water will have tissues more enriched in $^{18}O$ when grown at lower elevations and more southern latitudes compared to Pocatello. Alternatively, crops that integrate significant water from seasonally variable sources will have tissue $\delta^{18}O$ based on both season and location. Under that model, it becomes immediately evident that instances can occur where the differences in $\delta^{18}O$ due to changes in crop location are predicted to disappear due to seasonality if plant foods grown at more northern locations in summer are compared to similar foods from more southern locations in winter, as was done in this study (Fig. 1.8). This situation occurs because higher elevation northern locations like Pocatello have their most enriched $\delta^{18}O$ values for the year in the summer, and more southern (and low elevation coastal) locations have their least enriched $\delta^{18}O$ values in the winter. This implies that a seasonal comparison winter-grown and summer-grown crops may have no difference in $\delta^{18}O$ because the geographic location of cultivation changed. Thus, if seasonality matters, the effect of an annual north-to-south “snow birding” of leafy greens in Pocatello’s retail market would potentially result in little or no change in $\delta^{18}O$ between winter and summer.

Patterns of $\delta^{18}O$ variation observed in a subset of unverified leafy greens that provided crop cultivation location data at the point of sale (Table 1.3) confirmed the potential for combined effects of season and location (Table 1.5). For example, the site of ID1 spinach cultivation showed a purportedly large change in location (8.648° latitude, 0.7548° longitude), yet leaf $\delta^{18}O$ was virtually unchanged (0.21‰). This could be due to the confounding effects of crops grown in two different seasons at two different
locations. For ID1 lettuce, the site of cultivation changed very little between winter and summer samples (1.039° latitude, 0.233° longitude), yet the winter crop $\delta^{18}$O was enriched by 8.7‰ (Table 1.5); however, $\delta^{18}$O shifted in the opposite direction predicted by variation in monthly precipitation during the calendar year (Figs. 1.7, 1.8). This outcome seems contrary to expectations unless the change in season of cultivation corresponded to change in crop irrigation patterns, from primarily groundwater in summer to more precipitation in winter (a reasonable expectation for crops sourced to California’s average annual climate). Regardless of specific interpretations, the range of observed patterns are consistent with the hypothesis that latitudinal variation in groundwater is not the sole or main determinant of $\delta^{18}$O in edible leafy greens.

Under $\delta^{13}$C analysis, means of crop samples ranged from -30.1 to -25.9 ‰ (Tables 1.6, 1.7). These values fall within the normal range for leafy greens, being C3 plants, of -35‰ to -20‰ (Dawson et al. 2002). This indicated that sampled agricultural plants were generally not chronically water stressed because they exhibit relatively negative $\delta^{13}$C values (Gebrekirstos et al. 2011). If plants are not undergoing water stress, their stomata remain open allowing evapotranspiration that potentially impacts fractionation of $\delta^{18}$O within plant biomass (Barbour 2007, Lawson and Blatt 2014). Higher evaporation may result in plant biomass $\delta^{18}$O to become more enriched (Barbour 2007). Fractionation due to evaporation from leaves may have also driven some $\delta^{18}$O values among plants grown at different latitudes closer to each other than originally expected. For example, leaf crops grown at more northern, higher elevation latitudes are expected to have relatively depleted $\delta^{18}$O, but summer evapotranspiration may cause $\delta^{18}$O enrichment at those very same locations. Given the observed differences in $\delta^{18}$O
among different vegetables (Fig 1.5), generalizing these effects to other plant crops is not advised. Seasonal effects on $\delta^{18}O$ in loose leafy greens may be especially strong because of their high potential for evaporative fractionation that varies by season.

Available store labels were used in tandem with the $\delta^{18}O$ analysis of unverified foods to examine seasonal variation in produce vendor location. Using this information, grocery store samples from ID1 and ID2, regardless of season, never appeared to originate within 161km (100 miles) of Pocatello. For example, ID1 samples of lettuce and winter kale came from the southern California area (Fig. 1.9A). While ID1 summer kale was labeled as “Product of USA”. ID2, on the other hand, did not appear to change source locations very much during the winter and summer months and remained in southern California for all but one summer kale sample (Fig. 1.9B). For MT1, migration patterns conformed to a mainly local produce supply (within 161km, 100 miles) during the summer months and shifted to California and Mexico during the winter (Fig. 1.9C). Thus, there is no evidence to reject the second hypothesis: retail leafy greens available to Pocatello consumers never becomes truly local even during the summer months. However, these trends depend on both where consumers shop and where they live. For example, consumers that live in Portland and shop at ID1 could expect their supply of summer produce to be seasonally local. Conversely, if consumers lived in Pocatello and shop at ID1, produce is never sourced within a 161km (100 mile) radius during the winter or summer months. Missoula produce supplies during the summer months stayed within this local food radius, except for summer spinach that was labeled “Northwest Grown”. In this sense, the supplies between MT1, a retail grocer, and the seasonal farmer’s markets in Missoula converged to be similar for a portion of the calendar year.
Relying on unverified store vendor location information to evaluate patterns of variation in plant food $\delta^{18}$O as described above undermines an original study goal to do exactly the opposite: use variation in $\delta^{18}$O to predict crop locations, including instances where location data are unavailable. Fresh plant produce labels may sometimes accurately indicate approximate geographic locations of crop production, but it can often remain unclear to the consumer where the food is from, except in very general terms (e.g., “Product of Mexico” or “Grown in USA”). The U.S. Food and Drug Administration has no strictly defined rules stipulating that vendors explicitly display the location of where the produce was grown. Some plant foods are only described as “distributed by” or “packed by”, which potentially disassociates the points of plant crop cultivation with the physical sites of wholesale distribution (U.S. Food and Drug Administration 2013). Geographic variation in water isotopes chemically bound into plant tissue biomass does provide an alternative analytical opportunity to help ascertain where plant foods were grown (Brunel et al. 1997), independent of retailer labels that may only provide a location at some point along the food system distribution chain. Use of stable isotopes, including $^{18}$O/$^{16}$O, to verify claims about a given food’s geographic authenticity is on the rise, but they rely on cross-referencing unverified samples against geographically verified databases (Camin et al. 2017). That type of database resource was lacking in the present study, but could potentially be developed in the future. The current study indicates that seasonal variation for isotopes of oxygen and most likely hydrogen (i.e., H$_2$O) should be integrated into any efforts to develop verified crop plant databases that seek to successfully model expected geographic variation in bound water of food crops throughout the year. Given the recent episodes of disease dissemination via leafy green
vegetables, such a database may be become increasingly important to help rapidly identify potential sites where infected crops are from.

Stable isotope analysis indicated that all the estimated δ^{18}O values were heavier than annual precipitation (Bowen and Wilkinson 2002); this was as expected. This isotopic frameshift was attributed to the composition of cellulose (a main component of leaf dry mass) being ~27‰ higher than precipitation source water due to oxygen isotopes exchanging with water and glyceraldehyde-3-phosphate (Flanagan and Ehleringer 1991). Other sources of water such as soil, ground or tap may also influence δ^{18}O variation in the plant (Inácio, et al 2015, Evaristo and McDonnell 2017). Although no fractionation occurs during water uptake by root systems, plant structure and cultivation conditions may also influence evaporation effects resulting in fractionation differences between plant species (Inácio et al. 2015, Jones, et al. 2019). Differences in plant food type in the present study proved to be significant across the eight vegetables varieties analyzed. However, there was no significant effect of crop type when the food samples were constrained strictly to leafy greens from grocery retailers, as in the unverified portion of the study, suggesting vegetables might fall into different physiological functional groups.

Another factor contributing to the additional 1.9 - 23.9‰ frameshift in δ^{18}O away from estimated annual precipitation values could be due to monthly variation in δ^{18}O from precipitation by geographic location (Feng et al. 2009). Less enriched δ^{18}O values occur with increasing latitude and also exhibit a larger range of seasonal variation each year (Feng et al. 2009, Inácio et al. 2015). Although the verified sample analysis did not include elevation as a significant factor in the analysis, other studies have shown its
importance in $\delta^{18}$O fluctuations (Bowen and Wilkinson 2002). Perhaps a larger elevation gradient was needed to interpret this factor in the present analysis.

**Conclusions**

Although store-to-store food migration patterns differed, one major tendency persisted: Idaho and Montana winter produce becomes decidedly less local and production sites converged much further south in both Pocatello and Missoula. Although Pocatello and Missoula are similar in both size and location, Missoula appears to have a much more prolific local leaf supply during the summer months. Both Missoula and Pocatello offer farmer’s markets, but in addition to a farmer’s market, Missoula also offers larger retail stores with local produce readily available from a variety of local farms that comprise the Western Montana Growers Cooperative (Fig. 1.10). Other than the few local retailers at the Pocatello Farmer’s Market, this culture and infrastructure appears to be lacking compared to Missoula. Although these intermountain west cities are similar in latitude and climate, they appear to have substantial differences with respect to in-season, truly local food opportunities.

The results from this study showed $\delta^{18}$O can potentially estimate general trends in plant food origin, and the approach may be further developed as an effective method of comparing the food system dynamics of two comparable locations. The study also showed that comparable cities or locations do not always have the same food system dynamics despite their apparent geographic and climatic similarities. The Pocatello region could potentially benefit from the development and integration of a local food system based on regional farms that reduce the field to fork distance to improve local
food availability to consumers. While local food production in both sister cities is constrained by north temperate climates in the winter, the successes of the food production model in Missoula suggest useful, untapped opportunities to improve food system resilience during the traditional growing season exist for Pocatello.
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https://doi.org/10.1093/biosci/bix125.


Figure 1.1. Geographic variation in δ¹⁸O in surface precipitation in North America, based on annual averages (Bowen 2017, Bowen and Revenaugh 2003, Welker 2000).
Figure 1.2. Geographic points of origin of verified samples from home gardens and regional farmer’s markets. Sample locations were selected to encompass Pocatello, Idaho and Missoula, Montana (stars), two comparable intermountain cities expected to rely on a similar plant food system supply infrastructure in the western United States.
Figure 1.3. Regression analysis for pilot study of verified samples. The relationship was significant and negative, with more enriched samples (higher $\delta^{18}$O) at more southern latitudes (p-value: 0.017).
Figure 1.4. Multiple regression analysis of verified samples comparing winter and summer $\delta^{18}$O values (p-value: $6.538 \times 10^{-16}$). Symbol size reflects estimated mean $\delta^{18}$O; samples more enriched in $\delta^{18}$O are represented with larger circles relative to depleted samples (smaller circles).
Figure 1.5. Box plot for verified δ\textsuperscript{18}O samples comparing individual plant type. Whiskers indicate minimum and maximum values; the box represents the interquartile range where the bold line represents the median. Vegetation type significantly explained a portion of the variation in δ\textsuperscript{18}O values (p-value: 1.75 x 10\textsuperscript{-7}).
Figure 1.6. Interaction plot of $\delta^{18}$O in edible leafy greens by season for two Idaho grocers (ID1 and ID2) and one Montana grocer (MT1). Lines connect mean $\delta^{18}$O values during the summer and winter months in each retail store. Larger “slopes” indicate that winter season leaves are more enriched during the winter and depleted during the summer. However, the strength of the effect varied by store.
Figure 1.7. Monthly precipitation curves for Pocatello, ID, Missoula, MT, Portland, OR, and Monterey County, CA (Bowen 2019, Bowen et al. 2005, Welker 2000).
Figure 1.8. Offset monthly precipitation curves from Figure 1.7 with potential winter crop locations, CA and OR, frame-shifted by six months to illustrate how seasonal comparisons of $\delta^{18}$O in winter crops from more southern latitudes or coastal locations can converge on similar $\delta^{18}$O values from summer crops grown at more northern latitudes at higher elevation. For Portland, OR, and Monterey County, CA, month 1 represents January; for Pocatello, ID and Missoula, MT, month 1 is July.
Figure 1.9. Location of retailer sources for leafy greens provided by two Idaho grocery stores (ID1 and ID2) and one Montana grocery store (MT1) during winter (blue circles) and summer (green circles) based on unverified in-store location data available to consumers. Sources with ambiguous or missing location labeling were excluded from the figure. In panel C), southern star, Pocatello, ID; northern star, Missoula, MT. MT1 had a seasonal retail grocery supply pattern that best fit the main “snow birding” pattern hypothesized to exist for intermountain west cities. Unlike Pocatello, MT1 also exemplified a retailer model where many fresh plant foods available in a grocery store setting became truly local in the strict definition of the term used in this study.
Figure 1.10. Western Montana Growers Cooperative locations. Missoula, MT marked with star. The preponderance of small farmers around the city enables a significant supply of local plant food during the traditional summer to fall growing season via farmer’s markets and MT1.
Table 1.1. $\delta^{18}$O composition of verified sample locations used to test for geographical variation in plant foods by latitude and longitude. Values for $\delta^{18}$O are means ± s.d. estimated from three sub-replicate samples of one locally grown plant food, except for those in the 2017 pilot study (gray box) where five sub-replicates per sample were analyzed. Estimated annual values are as per (Bowen, G. J. 2019; Bowen G.J., Wassenaar L.I. and Hobson K.A. 2005).

<table>
<thead>
<tr>
<th>Verified Sample</th>
<th>Location</th>
<th>Harvest Month</th>
<th>Latitude</th>
<th>Longitude</th>
<th>$\delta^{18}$O ± s.d.</th>
<th>Estimated Annual $\delta^{18}$O</th>
</tr>
</thead>
<tbody>
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<td>Kale</td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>15.8 ± 0.2</td>
<td>-14.3</td>
</tr>
<tr>
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<td>41.737</td>
<td>-111.834</td>
<td>21.4 ± 0.2</td>
<td>-14.3</td>
<td></td>
</tr>
<tr>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>14.4 ± 0.1</td>
<td>-14.5</td>
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<tr>
<td>Potatoes</td>
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<td>Arco, ID</td>
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<td>Pocatello, ID</td>
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<td>41.737</td>
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<td>-14.5</td>
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Table 1.2. Summary of ANOVA results for verified δ¹⁸O samples (p-value: 6.538 x 10⁻¹⁶). A p-value less than 0.05 is flagged with one star (*), a p-value is less than 0.01 is flagged with two stars (**), and a p-value is less than 0.001 is flagged with three stars (***)..

<table>
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Table 1.3. Unverified seasonal $\delta^{18}$O data as a function of plant type, longitude and latitude for two Idaho grocers (ID1 and ID2) and one Montana grocer (MT1). For the Idaho and winter Montana samples, three samples of each species were taken and three sub-replicate isotope samples were taken from each sample. For the summer Montana samples, one sample of each species was taken and three sub-replicate isotope samples were taken from that sample. Mean $\delta^{18}$O values of each species are listed with respective standard deviations. N/A, not available.

<table>
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<th>Unverified</th>
<th>Store</th>
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<th>Longitude</th>
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<td>Summer</td>
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<td>Kale</td>
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<td>Spinach</td>
<td>&quot;Northwest Grown&quot;</td>
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<td>Kale</td>
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<td>Romaine</td>
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<td>Spinach</td>
<td>N/A</td>
<td>N/A</td>
<td>25.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kale</td>
<td>39.766466</td>
<td>-122.0365</td>
<td>23.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kale</td>
<td>&quot;Product of Mexico&quot;</td>
<td>19.3 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.4. Summary of ANOVA results for the effects of season, store, and vegetation on \( \delta^{18}O \) values. There was no significant effect of crop type or season on \( \delta^{18}O \) values, but there was a significant interaction of store and season on \( \delta^{18}O \).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Test Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>( F_{1,4.09} = 5.51 )</td>
<td>0.0772</td>
</tr>
<tr>
<td>Random</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Type</td>
<td>( X_{1}^2 = 2.83 )</td>
<td>0.0895</td>
</tr>
<tr>
<td>Store: Season</td>
<td>( X_{1}^2 = 8.32 )</td>
<td>0.0039</td>
</tr>
</tbody>
</table>
Table 1.5. Differences in latitude, longitude and $\delta^{18}$O between winter and summer unverified produce. Absolute values are listed.

| Unverified | Sample | $|W-S|$ Latitude | $|W-S|$ Longitude | $|W-S|$ $\delta^{18}$O |
|------------|--------|----------------|----------------|----------------|
| ID1        | Lettuce | 1.039          | 0.233          | 8.7            |
|            | Spinach | 8.648          | 0.7548         | 0.2            |
|            | Kale    | N/A            | N/A            | 10.0           |
| ID2        | Lettuce | 0.012          | 0.043          | 2.1            |
|            | Spinach | N/A            | N/A            | 5.0            |
|            | Kale    | 8.363          | 1.200          | 1.9            |
| MT1        | Lettuce | N/A            | N/A            | 7.8            |
|            | Spinach | N/A            | N/A            | 1.9            |
|            | Kale    | 6.644          | 7.878          | 0.1            |
Table 1.6. Verified sample $\delta^{13}C$ means ± s.d. estimated from three sub-replicate samples of locally grown plant foods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Harvest Month</th>
<th>Latitude</th>
<th>Longitude</th>
<th>$\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale</td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>-30.0 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>-30.0 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>-27.7 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Victor, MT</td>
<td>September</td>
<td>46.4105</td>
<td>-114.159</td>
<td>-27.7 ± 0.02</td>
</tr>
<tr>
<td>Spinach</td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>-27.7 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>-29.9 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Missoula, MT</td>
<td>March</td>
<td>46.8721</td>
<td>-113.994</td>
<td>-27.7 ± 0.04</td>
</tr>
<tr>
<td>Beet Leaves</td>
<td>Pocatello, ID</td>
<td>July</td>
<td>42.8237</td>
<td>-112.389</td>
<td>-26.6 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Missoula, MT</td>
<td>July</td>
<td>46.8632</td>
<td>-114.066</td>
<td>-26.2 ± 0.05</td>
</tr>
<tr>
<td>Red Tinge Lettuce</td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>-27.5 ± 0.03</td>
</tr>
<tr>
<td>Kohlrabi</td>
<td>Capay, CA</td>
<td>March</td>
<td>38.7061</td>
<td>-122.143</td>
<td>-29.0 ± 0.02</td>
</tr>
<tr>
<td>Mustard Greens</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>-29.4 ± 0.02</td>
</tr>
<tr>
<td>Butter Head</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>-28.8 ± 0.02</td>
</tr>
<tr>
<td>Arugula</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>-28.2 ± 0.02</td>
</tr>
</tbody>
</table>
Table 1.7 Unverified sample $\delta^{13}$C means ± s.d. estimated from three sub-replicate samples of one locally grown plant food.

<table>
<thead>
<tr>
<th>Unverified Store</th>
<th>Season</th>
<th>Sample</th>
<th>Latitude</th>
<th>Longitude</th>
<th>$\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID1</td>
<td>Summer</td>
<td>Green Leaf Lettuce</td>
<td>35.244737</td>
<td>-118.9153</td>
<td>-27.3 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>45.408672</td>
<td>-122.4964</td>
<td>-28.8 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>N/A</td>
<td>N/A</td>
<td>-30.1 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>Green Leaf Lettuce</td>
<td>34.205663</td>
<td>-119.1483</td>
<td>-25.9 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>36.761125</td>
<td>-121.7416</td>
<td>-29.3 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>34.205663</td>
<td>-119.1483</td>
<td>-28.4 ± 0.03</td>
</tr>
<tr>
<td>ID2</td>
<td>Summer</td>
<td>Romaine</td>
<td>36.6777</td>
<td>-121.6555</td>
<td>-28.3 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>N/A</td>
<td>N/A</td>
<td>-29.8 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>45.208245</td>
<td>-122.7381</td>
<td>-27.0 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>Green Leaf Lettuce</td>
<td>36.689714</td>
<td>-121.613</td>
<td>-25.9 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>35.173482</td>
<td>-120.5284</td>
<td>-29.3 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>36.8455</td>
<td>-121.538</td>
<td>-28.4 ± 0.03</td>
</tr>
<tr>
<td>MT1</td>
<td>Summer</td>
<td>Romaine</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>-27.7 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>N/A</td>
<td>N/A</td>
<td>-27.5 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>-27.5 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chard</td>
<td>46.8721</td>
<td>-113.994</td>
<td>-28.6 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple Kale</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>-29.7 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cabbage</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>-26.1 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bell Pepper</td>
<td>46.41605</td>
<td>-114.146</td>
<td>-28.2 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cauliflower</td>
<td>46.41605</td>
<td>-114.146</td>
<td>-27.1 ± 0.03</td>
</tr>
</tbody>
</table>
Abstract

Synthetic fertilizers have increased agricultural yields to keep up with growing world populations. However, synthetic fertilizers have detrimental impacts on the environment and organic agriculture is a suggested method of reducing such impacts. Stable isotope analysis (i.e., $\delta^{15}$N) was performed on samples collected from farmer’s market/local gardens, and Pocatello, ID and Missoula, MT local groceries. We tested the hypothesis that organic nitrogen sourced lettuce, spinach and kale would occur more frequently during summer months due to supply from locally sourced foods from smaller farms. Unpaired, two-tailed t-tests showed that ID1 and ID2 $\delta^{15}$N data partially support our hypothesis for lettuce, spinach or kale. Collectively, the $\delta^{15}$N results from this study provide preliminary evidence that some Pocatello retailers are sourced by farms that rely on a variety of nitrogen sources which may include synthetic fertilizers to manage crop productivity. Conversely, Missoula showed evidence of having predominantly organically sourced summer crops.
**Introduction**

Nitrogen is vital for nucleic acids and protein production, both of which are essential for plant growth (Canfield et al. 2010). However, not all forms of nitrogen found in the environment are available for these essential biological processes. To help solve the problem of nitrogen limitation in modern agricultural systems, the industrialized method for fixation of N\(_2\) was developed through the Haber-Bosch process. This process converts unusable N\(_2\) gas into useable forms such as ammonium (NH\(_3\)) (Willem et al. 2013). Nitrogen fixation processes have doubled the available amount of usable nitrogen compared to preindustrial levels, leading to unprecedented increases in agricultural production by application of synthetic fertilizers (Bouwman et al. 2009, Canfield et al. 2010). Although synthetic fertilizers are one factor that helped to increase food production by fifty percent since 1960, worldwide population is projected to further increase by another fifty percent by 2050 (Muller et al. 2017, Pretty et al. 2018). However, the amount of nitrogen applied in agriculture is about ten times the amount that is actually used by agricultural crops (Willem et al. 2013). Excess agricultural anthropogenic nitrogen contributes to nitrate (NO\(_3^-\)) deposition into ground water, and nitrogen oxides (NO\(_x\)), and ammonia (NH\(_3\)) emissions into the atmosphere (Willem et al. 2013). This anthropogenic waste can ultimately cause biodiversity loss, denitrification, and large releases of greenhouse gas (N\(_2\)O) into the atmosphere (Canfield et al. 2010, Pretty et al. 2018, Knapp and Heijden 2018). As non-organic agriculture provides 98.9% if the world’s food, anthropogenic nitrogen remains a large environmental issue (Schlesinger 2009, Tal 2018).
In order to combat these detrimental effects and feed a growing population, alternative methods to increase agricultural yields are often used. With respect to nitrogen fertilization, an alternative is greater reliance on organic fertilizers such as farmyard and green manure (Mie et al. 2017). Although organic methods of nitrogen supplementation often result in 30-40% reduction in yields compared to farming methods that rely on Haber-Bosch, the lack of synthetic fertilizers involved can avoid detrimental oversupply of nitrogen into the environment (Maeder et al. 2002, Muller et al. 2017, Knapp and Heijden 2018). In addition to decreasing anthropogenic nitrogen, organic soils tend to decrease deadly pathogens by fostering insect and microbe diversity (Jones et al. 2019). This is one of many ecosystem services that healthy soils can provide.

Organically sourced nitrogen additions to agricultural crops is one component of organic agriculture that is suggested to help transition food systems into more environmentally friendly practices (Knapp and Heijden 2018). While there are vague regulations on what can be labeled as “local” produce, the United States Department of Agriculture (USDA) “organic” label is highly controlled. Farmers must have an accredited certifying agent, pay allotted fees and undergo inspections in order to claim a “USDA Organic Certified” label (USDA Becoming a Certified Operation- Agricultural Marketing Service 2018). Although organic produce undergoes rigorous steps to be labeled as “USDA organic” it can still often be unclear or misleading to the consumer, due to products not being labeled as “USDA organic”, but being grown under organic methods of production. For example, produce may be cultivated under “organic” practices but if the land has not been chemical free for three years, produce cannot be
In order to characterize a representative sample of commercial plant foods available to consumers, retail leafy greens available in Pocatello, ID and Missoula, MT groceries as well as farmer’s markets and local gardens were sampled to analyze apparent crop nitrogen sources from isotope analysis. Nitrogen isotope ratios ($\delta^{15}\text{N}$) in plant biomass potentially support claims for organically sourced nitrogen verses synthetically grown produce (Rogers 2008). Previous studies have shown $\delta^{15}\text{N}$ can be correlated with methods of agricultural production, although it cannot solely discriminate nitrogen cultivation methods (Bateman and Kelly 2007, Rogers 2008, Mihailova et al. 2014, De Clercq et al. 2015, Serret et al. 2018). In this study, $\delta^{15}\text{N}$ was quantified for sampled leafy greens. As the Haber-Bosch process produces ammonium, there are many combinations of synthetic fertilizer compositions which results in various isotopic compositions (Bateman and Kelly 2007). Synthetic nitrogen fertilizers have a $\delta^{15}\text{N}$ value between -2 and 4‰ due to being sourced from urea (0.18 ± 1.27‰), ammonium (-0.91 ± 1.88‰) and/or nitrate (2.75 ± 0.76‰) (Kendall and McDonnell 1998). Organic fertilizers reportedly range from 2-30‰ due to their wide range of compositions and diverse origins (i.e., manure range from 5.3 to 7.2‰ and composts range from 9.3 to 20.9‰) (Bateman and Kelly 2007, Kendall and McDonnell 1998, Inácio et al. 2015).

While both synthetic and organic fertilizers exhibit these ranges, crop biomass $\delta^{15}\text{N}$ values vary based on factors like the timing of applying fertilizer, amount of precipitation, biochemical/physiological process, and the presence of mycorrhizae (Amundson et al. 2003, Bateman et al. 2007, Craine et al. 2009). As many mechanisms...
influence plant biomass $\delta^{15}$N, such analysis may not provide indisputable evidence about specific nitrogen sources (Bateman et al. 2007). Nevertheless, a survey of $\delta^{15}$N in retail plant foods has the potential to help corroborate whether specific crops are likely to be organically grown or not.

At the outset of this study, cultivation sites of lettuce, spinach and kale were expected to be “migrating” to more southern latitudes during the winter months. It was hypothesized that when sites of production moved south during winter months, large commercial farms would be more likely to be fertilized with synthetic nitrogen (more depleted $\delta^{15}$N). Conversely, during the summer months, produce would become locally sourced to smaller farm operations and fertilized with organic fertilizer with more enriched $\delta^{15}$N. The possibility of mixed N sources arising through combined application of synthetic and organic fertilizers could not be addressed with this approach.

**Materials and Methods**

Samples collected from farmer’s markets and local gardens will be referred to as “market” samples. Market samples were outside of the mainstream commercial supply chains used by retail grocery chains. Some of these market samples were known with high confidence to be fertilized with organic nitrogen sources (e.g., through direct farm visits and/or interviews). Retail plant food samples were those purchased from commercial retail grocery stores. Some retail grocery store samples were labeled with specific cultivation data (i.e., organic or homegrown) and others were lacking any specific label of cultivation methods. When available, cultivation methods from product labels was recorded.
For the retail facet of the work, three twist-tied bunches of retail spinach leaves, three heads of green leaf lettuce/romaine lettuce, and three twist-tied bunches of kale from two southeastern Idaho grocery stores (ID1 and ID2) were purchased at two times of year. The winter season purchase was on March 18, 2018 and the summer season purchase was on June, 15, 2018. Summer samples of chard, cauliflower, bell pepper, and purple kale were also obtained from a grocery store in Missoula, MT (MT1) on September 16, 2018. MT1 samples included crops described as “homegrown”; local growers in the Missoula area assert homegrown produce is organic, but not certified by USDA. Winter MT1 samples were not available for this study.

Samples from Idaho were rinsed with distilled water, patted dry, and stored in sealed plastic bags at ~ -8 °C until they were processed for analysis. Samples were oven dried at 50-70° C then hand-ground by mortar and pestle. Nitrogen stable isotope analysis was performed on three sub-replicate samples taken from each market sample. For retail samples, three sub-replicate isotope samples were taken from one randomly selected spinach, lettuce and kale sample from each both ID1 and ID2.

Samples for nitrogen elemental concentrations and stable isotope ratios were analyzed at the Idaho State University Interdisciplinary Laboratory for Elemental and Isotopic Analysis (ILEIA) using a Costech ECS 4010 elemental analyzer interfaced to a Thermo Delta V Advantage continuous flow isotope ratio mass spectrometer. Isotopic values are reported in the conventional δ-notation (δ = ([Rsample/Rstandard] – 1) * 1000, where R = \(^{15}\text{N}/^{14}\text{N}\)) relative to the international standard atmospheric N\(_2\) (air), and expressed as per mil (‰). Analytical precision, calculated from analysis of standards
distributed throughout each run, was \( \leq \pm 0.2\% \) for nitrogen stable isotopes, and \(< \pm 0.5\% \) of the sample value for \( \%N \).

**Statistical Analysis**

Retail samples were analyzed with unpaired, two tailed t-tests. These t-tests were used to compare mean \( \delta^{15}N \) seasonal differences within store (ID1 and ID2) for lettuce, spinach and kale. Nitrogen content was analyzed with unpaired, two-tailed t-tests for pooled market and retail lettuce, spinach and kale. The relationship between lettuce, spinach and kale nitrogen content with \( \delta^{15}N \) was analyzed with regression analyses for pooled market and retail samples. T-tests and regressions were done using GraphPad Prism version 8.0.2 for Mac OS X, 2019.

**Results**

Retail ID1 lettuce remained within the organic fertilizer range (i.e., 2-30\%) across summer (4.1\( \pm 0.04\)%) and winter months (6.10\( \pm 0.02\)%), although \( \delta^{15}N \) was \(~2\%\) more enriched in winter (p-value: 0.0001). ID1 spinach shifted from the organic range during the summer (12.8\( \pm 0.04\)% to the synthetic nitrogen fertilizer range (i.e., -2 to 4\%) during the winter (-1.5\( \pm 0.06\); p-value: 0.0001), which was consistent with the hypothesis. No seasonal change in \( \delta^{15}N \) was observed in ID1 kale; this crop remained within the organic range during both the summer (9.7\( \pm 0.01\)% and winter months (9.9\( \pm 0.12\); p-value: 0.1402). In the second retail grocer, ID2 lettuce shifted from the organic range during the summer (5.9\( \pm 0.01\)% to the synthetic nitrogen fertilizer range during the winter (-0.9\( \pm 0.01\); p-value: 0.0001) which was also consistent with the hypothesis.
ID2 spinach $\delta^{15}$N in summer was within the organic range (8.5± 0.03‰) and winter samples were significantly more depleted (2.3± 0.03‰; p-value: 0.0001). Wintertime $\delta^{15}$N in ID2 winter spinach leaves fell within the overlapping range of organic and synthetic $\delta^{15}$N values (i.e., from 2 to 4‰). ID2 kale was within the synthetic nitrogen fertilizer range (0.7± 0.02‰) but the winter sample was significantly more enriched and within the organic range (12.8± 0.06‰; p-value: 0.0001). In this instance, the winter crop was specifically labeled as “organic” at the point of sale, but the summer crop was not.

MT1 retail crops labeled as “homegrown” had values that ranged from 6.0± 0.04‰ to 11.3± 0.1‰ that all fell definitively within the organic fertilizer range. An unlabeled spinach sample with a $\delta^{15}$N value of 3.3± 0.03‰, fell within the overlapping range of conventional and organic fertilizers (2 to 4‰). This was the only crop that was not certified by the MT1 retailer to be grown in Montana. All the other unlabeled samples fell within the organic range from 4.8± 0.12‰ to 10.0± 0.1‰ (Table 2.1)

Market samples that were identified as organic had $\delta^{15}$N plant biomass values ranging from 2.0± 0.02‰ to 18.9± 0.04‰ (Table 2.2), which lies within the 2-30‰ organic fertilizer range, but includes some relatively depleted $\delta^{15}$N values that overlap with potential use of synthetic nitrogen. Market samples with unknown cultivation methods had samples that ranged from 6.4± 0.11‰ to 10.5± 0.03‰, well within the expected organic fertilizer range. In retail samples, organically labeled crops ranged from 4.1± 0.04‰ to 12.8± 0.06‰ and homegrown samples ranged from 6.0± 0.04‰ to 11.3± 0.1‰ (Table 2.1), both of which lie within the 2-30‰ organic fertilizer range. Retail leafy greens that were not expressly labeled as homegrown or organically grown had
δ^{15}N between -1.5 ± 0.06‰ to 10.0 ± 0.1‰. A range in δ^{15}N between -2 to 30‰ in plant dry mass was expected if a mixed cohort of retailer crops sourced to different farms received either synthetic, organic, or a combination of nitrogen fertilizer sources during cultivation.

Nitrogen content in dry mass of market samples across winter and summer ranged from 2.1 ± 0.1% (kohlrabi) to 6.1 ± 0.1% (arugula). There did not appear to be significant differences in nitrogen content between seasons as pooled retail and market lettuce samples ranged from 3.7 ± 0.8% during the summer and 4.0 ± 0.12% during the winter (p-value: 0.5899). Pooled retail and market spinach during the summer months ranged from 4.5 ± 1.0% to 5.4 ± 0.5% during the winter months (p-value: 0.1105). Pooled retail and market kale samples ranged from 3.8 ± 1.9% during the summer to 5.0 ± 0.7% during the winter (p-value: 0.2212). Collectively, there was no correlation when comparing leaf nitrogen and δ^{15}N in kale (R^2= 0.0255), spinach (R^2= 0.0116) or lettuce (R^2= 0.1175) (Fig. 2.1).

**Discussion**

The patterns of δ^{15}N in leafy green biomass observed in this study often supported cultivation claims, confirming that organically grown crops generally received organic nitrogen fertilization. Since plants potentially fractionate nitrogen during uptake, δ^{15}N in crop biomass may differ to varying degrees from δ^{15}N in fertilizer at the time of agricultural application. Thus δ^{15}N cannot always serve as the sole determinant of methods of nitrogen fertilization (Camin et al. 2011). In particular, it can be problematic to clearly verify if a crop has been organically fertilized when the nitrogen isotope ratio is
at the depleted end of the organic range that potentially overlaps with synthetic $\delta^{15}$N values (i.e., 2 to 4‰). Although $\delta^{15}$N alone does not always provide unequivocal evidence, this method still effectively discriminated many organic and inorganic vegetables whose $\delta^{15}$N values aligned with the cultivation claims made by the producer (Bateman et al. 2007, Bateman and Kelly 2007, Rogers 2008).

For this study, elemental stable isotope analysis was paired with information deduced from food labels and/or grocer/grower interviews, when available. It was hypothesized that when sites of production “migrated” south during the winter, large commercial farms at lower latitudes would use synthetic nitrogen sources with values between -2 and 4‰ (Kendall and McDonnell 1998). Alternatively, smaller “family” farms at higher latitudes that supply crops in summer would use fertilizers with higher $\delta^{15}$N (Bateman and Kelly 2007, Kendall and McDonnell 1998, Camin et al. 2011). Nitrogen fractionation in Pocatello’s retail leafy greens partially supported this hypothesis since three out of the six pairwise crop comparisons in Idaho retail grocers showed evidence of a shift to Haber Bosch nitrogen sources in winter. However, the use of synthetic fertilizer sources may ultimately depend on the farm vendor. Some crops did not exhibit synthetic nitrogen values in either season, even when they were not sold to consumers as certified organic. Conversely, ID2 summer kale appeared to receive synthetic nitrogen fertilizer and the winter crop was within the organic range. However, in this instance the retailer had labeled the winter crop as organically grown. In summary, there was some evidence to support the notion that ID1 and ID2 retail stores are more likely to rely on winter leafy greens that are fed synthetic nitrogen when compared to summer crops. However, consumer certainty about the use of organic nitrogen fertilizers
ultimately relies on certified organic labels at the point of sale. MT1 on the other hand, only had one sample that was within the overlapping range of synthetic and organic nitrogen fertilizers (spinach $3.3\pm 0.03\%$). This sample claimed to be “Northwest Grown”, so it likely was not from Montana. The rest of MT1 summer samples definitively fell within the organic range and were consistent with the hypothesis that during the summer months, produce that was locally sourced was fertilized with organic fertilizer.

Although $\delta^{15}N$ offered mixed results in comparing stores, it provided support for individual market and retail claims from all stores with known/labeled cultivation methods (i.e., organic or homegrown). These known/labeled samples all exhibited high $\delta^{15}N$ values that appeared to support producer cultivation claims (Table 2.1 and 2.2). In one case, a highly enriched market sample of beet leaves ($\delta^{15}N =18.9\%$) was from a small organic farm in Missoula, MT. After further investigation (i.e. interviewing the farmer), it was found that the sample was fertilized with cow manure. These highly enriched $\delta^{15}N$ values were presumably due to the evaporation of ammonia gas off organic manure resulting in higher $\delta^{15}N$ in residue (Rogers 2008). Thus, prudent management of the nitrogen cycle is important to mitigate nitrogen emissions, even in the setting of organic farming.

Along with $\delta^{15}N$, leaf nitrogen content was investigated. Lettuce, spinach and kale percent nitrogen did not exhibit significant differences between seasons, nor were there significant relationships between $\delta^{15}N$ and average leaf nitrogen content (Fig. 2.1). As the leaf nitrogen content did not change with $\delta^{15}N$, it appears as though organic and synthetic fertilizers do not impact leaf percent nitrogen.
Conclusions

A survey of $\delta^{15}$N in retail plant foods has the potential to help corroborate whether specific crops are likely to be organically grown or not. ID1 and ID2 trends lend partial support to the hypothesis that summer farm locations are more likely to use organic fertilizers. Locally grown MT1 summer produce exhibited $\delta^{15}$N values within the organic range. When paired with labeled cultivation data, $\delta^{15}$N served as an effective method to authenticate cultivation claims (i.e., organic or homegrown). Also, when looking at the relationship between percent nitrogen and $\delta^{15}$N, there was no relationship between fertilizer type and percent nitrogen within plants. Collectively, the $\delta^{15}$N results from this study provide preliminary evidence that some Pocatello retailers are sourced by farms that rely on a variety of nitrogen sources which may include synthetic fertilizers to manage crop productivity. As synthetic fertilizers may be a short-term economic solution, it ignores the suite of longer-term ecological costs associated with anthropogenic nitrogen additions.

While certain crops have lower yields under organic methods of production, advocacy of switching to solely organically sourced methods of agriculture (i.e. reliance on organically sourced nitrogen) can be controversial (Tal 2018). However, strategies that include reductions in food waste, larger land areas and changes in animal feeding rules, organic agricultural methods potentially provides sufficient food availability to address a growing population under sustainable methods (Muller et al. 2017). Greater reliance on sustainable agriculture can help reduce these impacts on biodiversity loss, denitrification and releases of greenhouse gas (Canfield et al. 2010, Tal 2018, Pretty et al. 2018).
Missoula, MT incorporates organic practices by offering “homegrown” local produce to consumers. For small farmers that do not produce a substantial amount of produce, obtaining strict and costly certification as “USDA Organic” can act as a deterrent to gain this certification. Furthermore, local growers’ participation in the local, organically grown food system in Missoula often predates the organic certification program established in 1990 by USDA. A term “homegrown” is used within the area to help a local cooperative (Our Farmers 2019) of farmers avoid costs of becoming USDA certified organic while allowing growers to sell their organic produce to consumers without USDA labels. This group of local farmers, established in fall of 2005, are within a 120km (75-mile) radius of Missoula, MT. Under this Homegrown Union, education is promoted and food is grown sustainably and locally (Montana Sustainable Growers Union- Homegrown 2018). Other than the few local retailers at the Pocatello Farmer’s Market, this culture appears to be lacking compared to Missoula. Although these intermountain west cities are similar in latitude and climate, they appear to have vastly different local food system opportunities.

Although two vegetables may appear outwardly similar, their pre-market cultivation can have vastly different impacts on the environment. Aware consumers can help combat these effects by choosing vegetables produced using methods that will more likely to be sustainable. The average consumer obviously cannot subject their produce to elemental analysis to help determine the $\delta^{15}$N values of their food if cultivation information is not provided. Therefore, consumers often lack the opportunity to choose produce grown under sustainable methods unless the food is clearly labeled as organic. An implicit goal of the current analysis is to help consumers gain insight on how nitrogen
in their foods come from different sources, and potentially highlights sources with a higher impact on the environment.
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Figure 2.1. Percent nitrogen verses $\delta^{15}$N plotted values for market and retail plant samples as well as trend per plant type.
Table 2.1. Retail sample means of $\delta^{15}\text{N}$ ± s.d. and %N ± s.d. Each mean was estimated from three sub-replicate samples from one plant purchased from a retail grocery store.

<table>
<thead>
<tr>
<th>Retail Store</th>
<th>Season</th>
<th>Sample</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Label</th>
<th>$\delta^{15}\text{N}$</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID1</td>
<td>Summer</td>
<td>Green Leaf</td>
<td>35.244737</td>
<td>-118.9153</td>
<td>Organic</td>
<td>4.1 ± 0.04</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>45.408672</td>
<td>-122.4964</td>
<td>Organic</td>
<td>12.8 ± 0.04</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>9.7 ± 0.01</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>Green Leaf</td>
<td>34.205663</td>
<td>-119.1483</td>
<td></td>
<td>6.1 ± 0.02</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>36.761125</td>
<td>-121.7416</td>
<td></td>
<td>-1.5 ± 0.06</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>34.205663</td>
<td>-119.1483</td>
<td></td>
<td>9.9 ± 0.12</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>ID2</td>
<td>Summer</td>
<td>Romain</td>
<td>36.6777</td>
<td>-121.6555</td>
<td></td>
<td>5.9 ± 0.01</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>8.5 ± 0.03</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>45.208245</td>
<td>-122.7381</td>
<td></td>
<td>0.7 ± 0.02</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>Green Leaf</td>
<td>36.689714</td>
<td>-121.613</td>
<td></td>
<td>-0.9 ± 0.01</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>35.173482</td>
<td>-120.5284</td>
<td></td>
<td>2.3 ± 0.03</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>36.8455</td>
<td>-121.538</td>
<td>Organic</td>
<td>12.8 ± 0.06</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>MT1</td>
<td>Summer</td>
<td>Romain</td>
<td>46.41047</td>
<td>-114.1589</td>
<td></td>
<td>10.0 ± 0.1</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>3.3 ± 0.03</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kale</td>
<td>46.41047</td>
<td>-114.1589</td>
<td></td>
<td>4.8 ± 0.12</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chard</td>
<td>46.8721</td>
<td>-113.994</td>
<td>Homegrown</td>
<td>11.3 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple Kale</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>Homegrown</td>
<td>6.1 ± 0.05</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cabbage</td>
<td>46.41047</td>
<td>-114.1589</td>
<td>Homegrown</td>
<td>8.6 ± 0.05</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bell Pepper</td>
<td>46.41605</td>
<td>-114.146</td>
<td>Homegrown</td>
<td>6.0 ± 0.04</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cauliflower</td>
<td>46.41605</td>
<td>-114.146</td>
<td></td>
<td>5.2 ± 0.04</td>
<td>4.0 ± 0.1</td>
</tr>
</tbody>
</table>
Table 2.2. Means of $\delta^{15}$N ± s.d. and %N ± s.d. in market samples. Each mean was estimated from three sub-replicate samples from one plant purchased from a farmer’s market vendor or collected from a local garden.

<table>
<thead>
<tr>
<th>Market</th>
<th>Sample</th>
<th>Location</th>
<th>Harvest Month</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Label</th>
<th>$\delta^{15}$N ± s.d.</th>
<th>%N ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale</td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>9.3 ± 0.04</td>
<td>Organic</td>
<td>5.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>9.4 ± 0.17</td>
<td>Organic</td>
<td>5.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>10.5 ± 0.03</td>
<td>Organic</td>
<td>5.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Victor, MT</td>
<td>September</td>
<td>46.4105</td>
<td>-114.159</td>
<td>4.8 ± 0.12</td>
<td>Organic</td>
<td>2.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>10.0 ± 0.03</td>
<td>Organic</td>
<td>6.0 ± 0.1</td>
<td></td>
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<tr>
<td></td>
<td>Logan, UT</td>
<td>March</td>
<td>41.737</td>
<td>-111.834</td>
<td>6.4 ± 0.03</td>
<td>Organic</td>
<td>5.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missoula, MT</td>
<td>March</td>
<td>46.8721</td>
<td>-113.994</td>
<td>6.4 ± 0.11</td>
<td>Organic</td>
<td>5.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Beet Leaves</td>
<td>Pocatello, ID</td>
<td>July</td>
<td>42.8237</td>
<td>-112.389</td>
<td>9.5 ± 0.08</td>
<td>Organic</td>
<td>3.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missoula, MT</td>
<td>July</td>
<td>46.8632</td>
<td>-114.066</td>
<td>18.9 ± 0.04</td>
<td>Organic</td>
<td>3.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Red Tinge</td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>9.5 ± 0.1</td>
<td>Organic</td>
<td>4.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>Pocatello, ID</td>
<td>March</td>
<td>42.8769</td>
<td>-112.435</td>
<td>9.5 ± 0.1</td>
<td>Organic</td>
<td>4.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Kohlrabi</td>
<td>Capay, CA</td>
<td>March</td>
<td>38.7061</td>
<td>-122.143</td>
<td>2.0 ± 0.02</td>
<td>Organic</td>
<td>2.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Mustard Greens</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>8.8 ± 0.1</td>
<td>Organic</td>
<td>5.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Butter Head</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>7.6 ± 0.04</td>
<td>Organic</td>
<td>2.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Arugula</td>
<td>Pocatello, ID</td>
<td>October</td>
<td>42.8237</td>
<td>-112.389</td>
<td>6.9 ± 0.01</td>
<td>Organic</td>
<td>6.1 ± 0.1</td>
<td></td>
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</tbody>
</table>
This project sought to characterize and evaluate aspects of Pocatello, ID’s local food system with stable isotope analysis of $\delta^{18}$O, $\delta^{13}$C, and $\delta^{15}$N. Stable isotope analyses were applied to better understand if edible plant food (specifically leafy greens) is changing location from summer to winter months and to examine what cultivation methods were used. $\delta^{18}$O analysis of plant biomass provided the opportunity to use geographic patterns of precipitation to estimate approximate locations of retail produce. $\delta^{13}$C isotope analysis helped further characterized $\delta^{18}$O, showing that sampled agricultural plants are well watered which results in higher amounts of $\delta^{18}$O fractionation (Barbour 2007, Lawson and Blatt 2014). From these results, it is apparent that plant food origin is moving between summer and winter growing seasons, however the interpretations of this data based upon strictly geographic variation were complicated by food samples grown at different times of year. As many agricultural crops are watered with an unknown combination of sources such as precipitation and groundwater (Evaristo and McDonnell 2017, Maupin 2018), the available data cannot firmly determine specific geographic origin in plant food. The current analyses suggested at least two potential patterns: 1) crops remained in nearly the same geographic locations with favorable agricultural conditions year-round, or 2) crops moved significant geographic distances, to higher latitudes in summer and lower latitudes in winter. $\delta^{15}$N analysis of these samples also allowed characterization of fertilizer additions to leafy produce available to consumers. Collectively, $\delta^{15}$N data characterized that foods available to Pocatello consumers may be sourced by a variety of different sources due to a large variation in $\delta^{15}$N values. As these methods effectively characterized Pocatello’s food systems in
general terms, it has even greater potential when combined with cultivation labels and other current methods of isotope analysis which include multi-element, multi-isotope ratio analysis, and reference databases (Drivelos and Georgiou 2012, Bonello et al. 2018).

In order to evaluate Pocatello’s local food system, a comparable sister city in the Intermountain West was used to compare local food systems to compare differences and recognize any opportunities to improve Pocatello’s local food system. Under δ\textsuperscript{18}O analysis, it appeared as though retail produce available to Pocatello consumers never originated within 161km, while summer produce from Missoula’s farmer’s market and one retail store originated within a 120km radius of Missoula. As it turns out, Missoula, MT has a prolific supply of locally grown organic produce while Pocatello appears to be lacking in this area. Perhaps this is due to Missoula having a local cooperative that incorporates growers within 120km (75-mile) radius that allows farmers to offer their organically grown produce as “homegrown” rather than having to undergo costly inspections to have the USDA “organic” label (Our Farmers 2019). However, during the winter months, Pocatello and Missoula appeared to converge on produce available from California and Mexico. Rather than just lamenting that during the winter months both food systems seem to be deficient, efforts to develop local winter food production methods in these locations is suggested.

**Leafy Greens in Northern Winters**

Decreasing our reliance on the industrial food system supported by agribusiness by migrating back to local production and food supplies cannot naively rely on a one-size-fits-all strategy. Local farmer’s markets can potentially improve consumer access to
fresh local plant foods, but since these supplies are intrinsically regional, regional supply constraints become immediately evident. Many locations cannot support year-round availability of locally harvested edible plants. For example, consumers in cold temperate climates commonly might be forced to rely on stored summer harvests such as potatoes, winter squash, and home canning efforts for local winter foods. Alternatively, consumers can (and often must) resort to integrating foods that are in-season elsewhere and transported into their local consumer markets. Thus, location-dependent seasonal constraints can force many plant-eating consumers back into the very food system they seek to escape. Reinventing local food systems to mitigate or eliminate unique place-based constraints requires creative design solutions that depend on where you are. Collectively, such regional solutions could ultimately contribute to a more resilient food system with many useful structural design features like increased decentralization and redundancy, and added food biodiversity (Walker and Salt 2006, Hemenway 2009).

To produce local food sustainably during the winter months, many methods such as temporary hoop houses and greenhouses have been used to attempt to grow local produce, but these techniques rely heavily on the use of plastics. This is often referred to as plasticulture, or a method of growing vegetables using plastic (Lamont 2005). While this approach creates a microenvironment allowing for season extension into the winter months (Abate 2008), there is also a significant stream of non-renewable waste products that result from these practices (Lamont 2005). It is estimated 80% of the litter in the oceans is plastic and causes damages to marine ecosystems (Toloken 2017). Consequently, these techniques are not environmentally benign nor are they inexpensive. As such, they are considered non-starters for long-term sustainability.
By striving to integrate place-based solutions, motivated consumers can become reconnected with where their unprocessed plant food is from and how it is cultivated. This can help recover lost knowledge about how food is grown and become a bridge between food making, and the biology and ecology of plants. Renewed appreciation of edible plant food that originates locally as an organism from nature is an important step for establishing useful links between healthy diets and the sustainable ecological principles that ideally support them. This knowledge of the biological processes of plants combined with systems thinking can allow for local solutions for global concerns.

In considering ideal local winter food production ideas, efforts were focused further on fostering resilience and sustainability, and perhaps equally importantly, the amount of time people are all willing to invest in making it happen. This last criterion was a concern because suggested solutions rely on investing personal time. In light of these self-imposed constraints, a number of factors were given priority to develop a system for making winter produce available in Pocatello: 1) Scale: work below the small farm level in winter, 2) location: work in places the eater visits at high frequency (near home or work), 3) time: opportunistically integrate time-saving practices in cultural methods, and 4) infrastructure: keep cultural methods as simple and close to nature as possible. As food production scales down, large equipment such as tractors can be eliminated. At a smaller scale, local foods can be added to the food system without the need for environmentally taxing practices such as poorly targeted fertilizers and pesticides. By keeping the operation small scale, it can also be kept within immediate home range. This will allow for 1) easy access while preparing foods, 2) minor modifications to be made due to weather fluctuations. In turn, this saves time.
**Rationale for a Winter Design**

The intent of design is not strictly based on the traditional “season extension” practice to keep summer plants safe as fall freezes encroach, nor to start summer plants earlier than normal in during the spring. Both of these traditional practices are acknowledged as useful for adding days of cultivation to the primary summer season. The alternative goal here is to deploy a suitable habitat for plants with the full expectation that they will provide food by intentionally cultivating species through the coldest part of the year along the winter annual life history. There’s overwhelming evidence that protecting plants from direct exposure to the elements enables cold tolerant plant survival and growth, as long as a certain amount of light is available (Nearing and Nearing 1977, Coleman 1989). This has led to the widespread use of easy-to-use designs that use overhead plastic sheathing and plastic fiber row cover “fabric”. Compared to plasticulture, the flat glass designs implemented here conserve and potentially even recycle interior system water, are heavy enough to stay put during windstorms, and allow plants to intercept more low intensity winter light than plastic. When the production scale is reduced from farm to garden, a tipping point is reached where glass is cost effective. In terms of fostering the application of useful knowledge, this project endeavored to find ecological studies that impact plant phenotypes then see if those manipulations can be adapted to serve as advantageous manipulations within our winter applications.

This is where placed based designs come into play. Utilizing winter annual plant life histories, allows this “down” season to become more locavore (i.e. one who consumes local foods) friendly. Sowing seeds in late summer to mid-fall allowed seedlings to establish themselves before colder, harsher conditions continue to persist.
Once temperatures drop, 6.35mm (.25 inch) thick tempered glass propped up on a 120x120x38 centimeter (48x48x1.5 inch) wood frame. This design proved to provide a more suitable habitat. By striving to integrate place-based solutions, motivated consumers can become reconnected with where their unprocessed plant food is from and how it is cultivated. This can help recover lost knowledge about how food is grown and become a bridge between food making, and the biology and ecology of plants. Renewed appreciation of edible plant food that originates locally as an organism from nature is an important step for establishing useful links between healthy diets and the sustainable ecological principles that ideally support them. This knowledge of the biological processes of plants combined with systems thinking can allow for local solutions for global concerns.

And it works.
Winter annuals (spinach and mâche) photographed on February 27, 2019. Plants were grown under glass, outside, from seeds sown on October, 2018.
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