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*Utah State University*

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INCREASING THE SUSTAINABILITY OF UTAH FARMS BY INCORPORATING  
QUINOA AS A NOVEL CROP AND PROTECTING SOIL HEALTH

by

Kristine R. Buckland

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science

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Logan, Utah

2016

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## ABSTRACT

Increasing the Sustainability of Utah Farms by Incorporating Quinoa as a Novel Crop and  
Protecting Soil Health

by

Kristine Buckland, Doctor of Philosophy

Utah State University, 2016

Major Professor: Dr. Jennifer R. Reeve  
Department: Plants, Soils and Climate

Most of the western United States faces increasing water shortages in the coming years, which will prove a major challenge for maintaining sustainable farms.

Incorporating an alternative crop that is well adapted to the projected climate could be a successful approach to increasing the sustainability of farms in the region. Quinoa, *Chenopodium quinoa* Willd., may be an ideal alternative crop to meet the demands of the Intermountain West. Before widespread adoption of this novel crop can occur, best management strategies need to be documented. This paper provides research on cropping systems, irrigation rates, and weed competition with quinoa. Additionally, the impacts of prior cropping history and compost addition on soil health parameters are presented.

Quinoa responded to compost addition in an organic cropping system trial where low soil phosphorous was a limiting nutrient. Cover crops, 70% hairy vetch (*Vicia villosa* Roth.) and 30% winter wheat (*Triticum aestivum* L.), provided sufficient nitrogen inputs for the following quinoa crop. In response to a line source irrigation trial, varieties showed

optimal irrigation rate from 23- 42 cm of water for biomass accumulation, although no seed was produced by any variety. In a greenhouse weed trial, quinoa was less impacted by the presence of any other species, lambsquarters (*Chenopodium album* L.), red root pigweed (*Amaranthus retroflexus*) and green foxtail (*Setaria viridis*), suggesting a high competitive advantage. Finally, organically managed soil increased soil health indicators, including microbial biomass and resistance to stress, regardless of compost addition. In addition, compost increased soil health indicators in conventionally managed soil. Seed set across all field trials was hindered by peak summer temperatures above 32°C, a known temperature sensitivity threshold during flowering for the varieties tested. Therefore, further work to select adapted varieties for the region must be accomplished before widespread adoption is feasible. An integrated approach involving a locally-adapted novel crop and soil health protection promises to increase future farm sustainability.

(180 pages)

## PUBLIC ABSTRACT

Increasing the Sustainability of Utah Farms by Incorporating Quinoa as a Novel Crop  
and Protecting Soil Health

Kristine Buckland

Most of the western United States faces increasing water shortages in the coming years, that will present a major challenge for maintaining sustainable farms. Incorporating an alternative crop that is well adapted to projected changes in climate could be a successful approach to increasing the sustainability of farms in the region. Quinoa, *Chenopodium quinoa* Willd., may be an ideal alternative crop to meet the demands of the Intermountain West. Before widespread adoption of this novel crop can occur, best management strategies need to be documented. This paper provides research on cropping systems, irrigation rates, and weed competition with quinoa. Additionally, the impacts of prior cropping history and compost addition on soil health parameters are presented. Seed set across all field trials was hindered by peak summer temperatures above 32°C, a known temperature sensitivity threshold for flowering. Therefore, further work to select adapted varieties for the region must be accomplished before widespread adoption is feasible. An integrated approach involving a locally adapted novel crop and soil health protection promises to increase future farm sustainability.

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Kristine Buckland

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## CHAPTER I: GENERAL INTRODUCTION

Quinoa, *Chenopodium quinoa* Willd., has the potential to be an alternative crop to meet the demands of the Intermountain West. In this area, marginal soils with low nutrient availability, low organic matter, low soil moisture, and high salinity are common. Quinoa has been developed under low-nutrient input systems, with demonstrated drought and salinity tolerance (Geerts et al., 2008; Jacobsen, 2003; S. E. Jacobsen et al., 2003; A. Peterson and Murphy, 2015a; Sun et al., 2014). Climate modeling predicts widespread drought of increasing severity due to increased temperatures and decreased precipitation for much of the Western US region as the 21st century progresses (Gutzler and Robbins, 2010; Wehner et al., 2011). Poor soil conditions are likely to be exacerbated as drought conditions become more widespread in the future, challenging the sustainability of regional farms. Incorporating an alternative crop that is well adapted to the projected climate could be a successful approach towards meeting these challenges.

Quinoa is a traditional crop in South America, particularly in the regions around the Andes Mountains where subsistence farming is common (Jacobsen 2003; Bhargava et al., 2006). The grain is harvested and used in many local dishes because of its nutty flavor and exceptionally high nutrient content (Kozioł 1992; Bhargava et al., 2006; Jacobsen 2003). It is a gluten-free food and has become a desirable part of a health-conscious diets in many countries around the world (González et al., 2015; Wu, 2015). The demand for quinoa, mostly organically grown, has resulted in elevated prices with organic quinoa being sold for \$12 to 19 US kg<sup>-1</sup>, over five times the price seen in 2006 (Arco, 2015; DePillis, 2013). The demand and market value of quinoa have caused



intensification of production practices such as increased tillage, removal of nearby grazing fields for livestock and subsequent reduction of organic matter inputs (Jacobsen 2011; Arco, 2015). Intensification has resulted in increased erosion, soil degradation, and greater pest pressure which has rendered an ancient crop production system seemingly unsustainable (Jacobsen 2011; Arco 2015).

By providing a novel crop in rotation that adds diversity, tolerance to extreme climate conditions with a high market value quinoa may increase sustainability on farms in the western US. Sustainable farming is an integrated approach that balances five major aspects as defined by US Title 7 Section 3103; in short, satisfying food and fiber needs while maintaining economic and quality of life indicators with minimal environmental impact. A sustainable farming system relies heavily upon a robust crop rotation program (Altieri, 1999; Altieri and Letourneau, 1982a; Hole et al., 2005). Including a new crop into a rotation system requires a thorough, regionally-adapted understanding of plant nutrient and water requirements, growth characteristics and optimum management strategies.

Quinoa in rotation would provide increased crop diversification, and prove essential when considering future water shortages or increasing global temperatures. Diverse cropping systems have also been shown to increase the sustainability of farms by reducing pest pressure and increasing soil health (Wang et al. 2011; Abawi and Widmer 2000; Wang et al. 2003; DuPont et al. 2009). Novel crops have been used in other systems to interrupt ideal pest habitats, which reduces the need for chemical controls,

costs to growers, and risks to the environment (Pimentel et al. 1992; Liebman and Dyck 1993).

There are five major ecotypes of quinoa: Altiplano, Salares, coastal, valley and Yungas, which correspond to diverse environments in and around the Andes mountains of South America (Gomez-Pando, 2015; Martínez et al., 2015). Field conditions are frequently harsh in the regions around the Andes mountains, with little or no irrigation, saline soils, and large temperature fluctuations (S.E. Jacobsen et al., 2003). There are a handful of growers in the United States, mostly in southern Colorado, who have developed varieties for an arid mountain environment where strong winds and cooler summer temperatures prevail, similar to the Altiplano ecosystem (A. J. Peterson and Murphy, 2015). However, the hotter summers in Utah coupled with the salinity problems in local soils may require planting varieties that do not originate from the Altiplano. The salinity tolerance of Salares varieties or the heat tolerance of the coastal varieties may provide ideal crop attributes for Utah facing hotter, drier weather predictions (Gutzler and Robbins, 2010; Wehner et al., 2011).

Currently, there are few varieties available to growers in the US. While South American countries have between 2,500 and 16,000 accessions in seed banks, the USDA National Genetic Resources Program (GRIN) has less than 300 (Gomez-Pando, 2015; Rojas, 2003; Zurita-Silva et al., 2014). Little information is available for the majority of these varieties besides genetic mapping, which suggests region of origin. A breeding program and regional field trials have been initiated by Washington State University, with cooperators in Oregon and Utah. Preliminary evaluations point to pollen sterility or

seed abort at summer temperatures exceeding 32 °C during flowering (A. J. Peterson and Murphy, 2015). The identification of adapted varieties and management practices including harvest and post-harvest seed cleaning for commercial production of quinoa in the western US remains a significant impediment to widespread production.

In order to develop systems that include quinoa in rotation, specific areas of management need to be examined further. My research entails an organic intercropping trial, an irrigation rate study, and a weed competition trial to investigate multiple facets of particular importance in organic quinoa production. A more in-depth look at how common environmental stressors influence soil microbial biomass will also be completed to assess the impact of cropping history on soil resistance and resilience properties. The studies listed here are designed to gather essential information in order to establish quinoa management strategies appropriate for our region and in addition, quantify the impact of cropping history on soil resistance and resilience. The four areas of study will lay the foundation required for successfully integrating quinoa into the region while conserving scarce resources of water and soil organic matter and enhancing overall soil quality.

The overall goal of this research is to assess the potential for adoption of quinoa in Utah cropping systems. The specific objectives are to:

1. Evaluate the efficacy of three different organic cover-cropping systems on quinoa yield and soil quality indicators.
2. Determine response of 10 varieties of quinoa to a range of soil moisture conditions.

3. Quantify the impact of weed competition on quinoa growth.
4. Evaluate the impact of cropping system history on soil resistance and resilience.

## CHAPTER II: LITERATURE REVIEW

### History of quinoa

Quinoa has been cultivated for thousands of years in and around the Andes mountains in a variety of diverse ecosystems (FAO, 2011; Jacobsen, 2003; González et al. 2015). Although recent genetic analysis of modern varieties suggests multiple origins of evolution, quinoa is viewed as a native crop of the Andean cultures (Martínez et al., 2015). The varieties of quinoa fall into five major ecotypes: Altiplano, Salares, valley, coastal and Yungas (Martínez et al., 2015; Gomez-Pando 2015). The Altiplano ecosystem is a high mountain plane, with cooler temperatures and short growing seasons. The Salares describes the salt flats near Lake Titicaca. Coastal varieties are generally adapted to warmer temperatures, with less salinity or drought tolerance. Valley varieties have been selected for growth in warmer, more temperate climates. The Yungas ecotypes are probably the fewest in number and have emerged from years of adaptation to a jungle-type climate.

Quinoa has received much attention lately by both the media and researchers. The FAO declared 2013 as the “Year of Quinoa” in an effort to promote quinoa as a tool to increase worldwide food stability. Media sources including the Washington Post and the New York Times have highlighted some of the major benefits and controversies surrounding the surge in quinoa consumption (DePillis, 2013; Romero and Shahriari,

2011). As an ancient crop with deep cultural ties to the Andean countries of South America, research and production of quinoa is now spreading world-wide.

Bolivia and Peru have been the top two producers of quinoa for more than twenty years. These two countries alone have increased production between 4.5 to 7 % per year since the mid-1990s, and total worldwide production increasing from 20,000 to nearly 140,000 hectares (Arco 2015, FAO 2013). In contrast, the US estimated production in 2011 was a mere 3,000 metric tons (FAO 2013). According to the FAO, Bolivia and Peru export approximately 26 and 10 metric tons annually, respectively, comprising 96% of all exports. In 2013, the US imported the most quinoa, more than 3 times any other country, over 36,000 metric tons, with 25,000 of those certified organic (Arco, 2015).

While media attention has undoubtedly encouraged consumption within the US, much of the appeal is due to the unique nutritional content of quinoa. Quinoa is the only grain crop with a complete protein that is also gluten free (González et al., 2015; Koziol, 1992; Wu, 2015). It can be consumed in a similar manner to rice or cereal crops. When compared to rice, maize or wheat, quinoa has higher amino acid, mineral and protein content (Koziol, 1992). Also, much of the quinoa sold is produced organically, which adds to market value and desirability for health conscious consumers. The high nutritional value of quinoa is a primary reason the FAO has focused on it as a crop with potential to increase food security worldwide.

Though rising crop prices may seem beneficial, controversy has surrounded the rapidly growing market for quinoa. The crop is traditionally grown by small-scale

farmers on subsistence farms. Critics say the increased prices have encouraged small farms to sell their own rations, which tend to be replaced with less nutritious alternatives (Arco 2015; Jacobsen 2011). Per capita consumption in Bolivia has decreased since 2000, although not much change was noted in Peruvian consumption patterns (FAO 2013). According to Arco (2015), Bolivian subsistence farmers have benefited from an over four-fold market price increase which has allowed younger generations to leave the family farm and seek higher education. The high market value has also encouraged the intensification of farms, particularly in the high altitude salt flats of Bolivia, the Salares, where the climate is particularly harsh (Arco 2015; Jacobsen 2011). Here, in an effort to increase quinoa production, virgin land is put into production with great dependence on increased tillage frequency, reduced organic matter inputs and shorter crop rotation than traditionally used. The result is degraded soil, increased pest problems and reduced yields (Jacobsen 2011).

Clearly, the demand for quinoa has increased in the worldwide market and traditional production practices cannot feasibly meet the demands. Growers in the US have had limited success with quinoa production, mostly in the southern high plains of Colorado (Peterson and Murphy 2015). Recently, researchers at Washington State University in Pullman, Washington, have begun breeding programs to identify potential varieties for organic production in the western US, as much of the quinoa market is organic and diverse crop rotations are ideal in an organic cropping system (Peterson and Murphy 2015).

To find suitable varieties for successful cropping in the western US, plant breeders must rely on basic information about the suspected origin of the individual variety. Bolivia alone has over 5,000 accessions in a national germplasm bank (Gomez-Pando, 2015; Zurita-Silva et al., 2014). However, the United States Department of Agriculture Genetic Resources Information Network (USDA-GRIN) has approximately one-tenth as many accessions available. Selection based upon region of origin is difficult as many of these accessions have limited passport data that provides such detail. Christensen et al (2007) used genetic mapping on a wide range of these accessions to provide some information on lineage; however, many of these varieties have not been field tested, and even fewer have published results.

Similar to farms near the Andes, organic farms in Utah are also subject to diverse regional influences on growing conditions. However, the majority of existing farms are in the Salt Lake and Cache valleys where summer temperatures can exceed 37° C and rainfall is scarce in the summer months (Utah Climate Center Data). Soils have low SOM and tend to have saline conditions. These local conditions suggest quinoa varieties with origins in the Altiplano or Salares ecotypes may be the most successful in Utah. In order to increase the sustainability of local farms and combat the harsh climate conditions, growers look towards management practices that increase soil health, such as diverse crop rotations and incorporating organic matter. One approach to improving the sustainability of Utah farms could be through incorporating quinoa as a novel crop into organic cropping systems.



## Organic cropping systems

Organic growers must eliminate synthetic fertilizers and pesticides in order to comply with USDA National Organic Program regulations. Without these chemical inputs, organic farms rely on methods such as crop rotations, cover crops or organic inputs to provide nutrients and control pests. Organic growers frequently rely on compost additions to help meet crop nutrient requirements. However, compost is bulky and the cost to transport and apply can be prohibitive. Additionally, compost has a low available nitrogen (N) to phosphorus (P) nutrient ratio; therefore, crop N requirements cannot be supplied through compost alone without applying excessive P. In order to provide adequate nutrients, growers rely on several methods. Systems including nitrogen fixing cover crops, intercrops and green manure crops are some ways to enhance available nutrients for a cash crop, especially N (Tonitto et al. 2006).

Intercropped systems involve two or more agronomic crops in various spatial or temporal arrangements. The combination of crops is designed to increase field diversity which can lower pest pressure and increase nutrient acquisition through changes in root structure or function (Altieri, 1999; Altieri and Letourneau, 1982b; Betencourt et al., 2012; Zuo et al., 2000). Yield advantages have been reported in several different intercropping systems (Gao et al., 2009; Li et al., 2001; Zhang et al., 2008). However, there is frequently a trade-off in yield versus other benefits due to likely crop-intercrop competition. In a winter wheat/spring maize intercropping system, researchers demonstrated a significant yield advantage over monocultures, however water use efficiency was lower than with maize alone (Gao et al., 2009). Crop growth rates and N-

use patterns also differ between mono- and intercropping. For example, when intercropping wheat with cotton, Zhang et al. (2008) reported slower rate of N uptake by cotton, but similar physiological N-use efficiencies by both crops. The authors describe the differences between the timing and amount of N-use which suggests each intercropped system requires in-depth consideration to optimize production and ensure sustainable systems (Zhang et al., 2008).

As a result of interspecies interactions, nutrient accumulation in the cash crop can increase. While the uptake of N is frequently reported, the accumulation of other nutrients such as phosphorous, potassium and micronutrients have been shown to improve with intercropping as well (Xia et al., 2013; Zuo et al., 2000). In a calcareous soil, peanut uptake of iron, a frequently limiting nutrient, was increased when intercropped with maize (Zuo et al., 2000). In this same study, researchers observed changes in the rooting patterns of peanut; notably, a much deeper rooting system, similar to that of maize, was developed in the intercropped treatments, which likely had an effect on nutrient acquisition (Zuo et al., 2000).

Another way to increase nutrient availability for cash crops is by incorporating cover crops or green manure crops. Cover crops are generally grown after the cash crop and may be used to reduce soil erosion and reduce nitrate loss from the system (Baggs et al. 2000; Cherr et al. 2006; Eigenberg et al. 2002). Similarly, a green manure crop is usually a legume, which can increase plant available N through biological nitrogen fixation while reducing the chances of negative environmental impacts of N loss (Cherr

et al. 2006; Crews and Peoples 2004). Neither cover crops nor green manure crops can provide a full complement of nutrients required for a cash crop; therefore, a system which incorporates diverse cropping systems, cover crops, green manure and the addition of compost may be ideal.

### Soil Health

Soil health has been described as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA Natural Resources Conservation Service 2014). Management strategies to maintain soil health have been shown to decrease pest pressure, increase plant available nutrients and be environmentally beneficial (Altieri and Nicholls 2003; Abawi and Widmer 2000; Wang, et al. 2011; Crews and Peoples 2004). Maintaining healthy soils is a key component of organic agriculture and increases the sustainability of any cropping system.

Incorporating organic matter into the soil is a fundamental approach to maintaining soil health. Frequently, growers use compost applications. However, the excessive use of compost can increase P levels without adequate N. Nitrogen fixing green manure crops can provide a valuable addition of N with the added benefit of helping to improve weed control, decreasing soil erosion and reducing environmental concerns associated with production of synthetic N sources (Al-Khatib et al., 1997; Baggs et al., 2000; Jensen and Hauggaard-Nielsen, 2003; Malik et al., 2000).

Measuring soil health frequently involves a number of complementary indicator tests. These indicator tests are typically chosen to assess the physical, chemical and

biological characteristics of the soil. While no single test can accurately describe complex soil characteristics, comparing tests from these three major areas of soil function can offer useful insight into the nutrient availability, nutrient cycling and overall impact of different management strategies (Doran, 2002; van Bruggen and Semenov, 2000).

Soil physical measurements provide information about the size and structure of soil particles and aggregates that help determine key qualities such as pore space, infiltration, and moisture holding capacity. One key measure of physical structure is bulk density which is a measure of how tightly packed soil aggregates are in a given volume of space (Elliot et al., 1999). Bulk density has important implications on the available pore space for air or water as well as the ability of roots to penetrate the soil. Adequate pore space allows for gas exchange, critical for plant roots as well as soil microbes, and enables more efficient flow of solutes by diffusion and mass flow for nutrient uptake. Changes in bulk density can occur with mechanical disturbances such as tillage or with crops of different rooting structures (Brady and Weil, 1996). Similarly, aggregate stability provides insight into the impact of mechanical or biological processes on the structure of soil (Douglas and Goss, 1982). The formation of soil aggregates can be encouraged by active microbial populations, invertebrates or by root exudates (Brady and Weil, 1996; Voroney, 2007). A soil with stable aggregate structure is better able to hold pore space open under stress such as a rapid downfall of rain (Oades, 1984).

Soil chemical properties are frequently assessed to determine plant available nutrients. Soil extractable elements provide information on the availability of macro or

micronutrients in the soil at a particular time and can be used as a guide to estimate the nutrient status of a system (Magdoff et al. 1984; Arrobas et al. 2012). Soil chemical properties, like extractable ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ), show only a snapshot of the nutrients available at sampling time. Extractable soil  $\text{NO}_3^-$  at 30 days after planting has been used for crops with a high N demand such as corn, and may be indicative of available N for quinoa (Magdoff et al. 1984). However, these values are generally not reflective of available N over the course of the growing season or for future crops and tend to underestimate the long term benefits of organic matter inputs (Arrobas et al., 2012). The availability of nutrients may be more accurately complemented by biological tests aimed at determining the microbial activity which is responsible for the turnover of SOM, the major source of long-term nutrients within the soil (Arrobas et al., 2012).

Traditionally, measurements of soil microbial biomass and soil respiration have been used to assess the size and activity of microbial populations which play an important role in not only soil physical properties and nutrient turnover, but also disease and pest suppression (Abawi and Widmer 2000; DuPont et al. 2009). The use of enzyme assays have proven effective in indicating changes in soil health in response to stressors such as wet/dry cycles, nutrient limitations or physical disruptions (Aon and Colaneri, 2001; Doran and Zeiss, 2000; van Bruggen and Semenov, 2000). One enzyme,  $\beta$ -glucosaminidase, has been shown to be a good indicator of mineralization of N and C as well as disease suppression. In one field experiment, Ekenler and Tabatabai (2002) found correlations between  $\beta$ -glucosaminidase levels and N mineralization, microbial biomass and organic C and N content of soils. In the same study, soils with longer, more diverse

cropping histories had higher  $\beta$ -glucosaminidase activity than those without rotation or in monoculture (Ekenler and Tabatabai, 2002). This same enzyme has also been shown to suppress multiple plant fungal pathogens (Lorito et al. 1994).

Quantifying organic matter within the soil is also useful in evaluating the overall health of soil. Total N and organic carbon (C) are frequently used to measure the organic matter (OM) content of soils but these tests do not indicate if the OM is labile or recalcitrant. Soil respiration tests to determine the mineralizable C fraction can be used to show the potentially labile C content of a soil (McLauchlan and Hobbie, 2004). Another approach is to determine the particulate organic matter (POM) of a soil, an important fraction of the SOM. In general, POM represents a more labile portion of SOM which can be broken down readily by an active microbial population (Bending et al. 1998; Marriott and Wander 2006). Management practices such as intercropping, reduced tillage, or compost additions have been shown to increase POM (Janzen et al., 1992; Marriott and Wander, 2006).

Soil resistance and resilience are also major indicators of soil quality (Seybold et al., 1999). The terms soil resistance and resilience stem from ecological concepts and refer to the soil response to disturbances, such as heat or freeze events, tillage, or chemical contamination (Seybold et al., 1999; Abner and Melillo, 1991). Soil resistance describes the ability of a soil to continue to function without decline following a disturbance. Soil resilience describes the rate and degree of functional recovery of a soil following disturbance. Many factors can influence resistance and resilience, including

soil type and texture as well as cropping history. Agricultural soils are constantly subjected to disturbances from both cropping practices and natural processes.

According to Seybold et al. (1999), “biological communities, both above and below ground, are among the most significant factors affecting soil resilience.” Measurements related to the size and activity of soil microbial populations have been used to describe soil resistance and resilience to stress (Benitez et al., 2004; Kumar et al., 2014; Udawatta, 2010). Management practices such as compost additions and diverse cropping systems tend to build the size and health of microbial population while practices such as frequent tillage or repeated herbicide have been shown to alter the soil microbial community structure or function (Lancaster et al., 2010; Seghers et al., 2003; Zablotowicz et al., 2007).

### Irrigation

In many traditional cropping systems in South America, quinoa is grown without irrigation, referred to as dryland farming. However, in areas with irrigation available, seed production has been reported to increase up to 40% (Geerts et al. 2008). Response to drought stress is highly dependent on variety and developmental stage in quinoa. Drought stress during flowering or grain fill can reduce yields while drought stress during early vegetative stages (both 2-6 and 6-12 leaves stages) did not impact yields (Geerts et al. 2008). Additionally, drought stress early in the season can lengthen time to flowering but can also hasten maturity if stress occurs after flowering (Geerts et al. 2009, 2008).

Quinoa water use ranges from 0.52 to 1.00 times the reference evapotranspiration rate, depending on phenological stage (Garcia et al. 2003).

The efficiency of a cropping system serves as another useful measure of how plants respond to water stress. As a measure of cropping system efficiency, harvest index is frequently reported for crops. Harvest index is simply the ratio of useable yield to total plant yield or biomass. However, when evaluating plant community efficiency, harvest index is only one component. Overall plant community efficiency depends the ratio of energy input to the ratio of energy output (Monteith and Moss, 1977). The input into the system can be measured through light interception (LI) whereas the output is a combination of photosynthetic efficiency, respiration efficiency, and harvest index (Monteith and Moss, 1977). Under ideal growth conditions where nutrients and water are not limiting, LI by leaves has the greatest impact on cropping system efficiency, as the other three components are generally a function of genetics. During periods of water or nutrient stress, however, these four components can vary greatly (Flexas et al., 2006).

Light interception in the plant canopy is defined as the ratio of incoming photons of light to the quantity of photons that penetrate the canopy and hit the ground, and therefore do not provide any energy to drive photosynthesis. The ratio can be measured directly and changes with the amount and quality of sunlight and with the development of plant canopy structure. A highly correlated approximation of LI of a plant community can be obtained through digital photography. The ratio of green pixels to total pixels photographed has been reported to have a 1:1 correlation with LI (Gonias et al., 2012;



Purcell, 2000). This method is not sensitive to the position of the sun and can be verified through selective subsampling of photon capture using a portable quantum meter.

Furthermore, the time required to take samples is minimal and data can be stored for long periods of time for analysis when convenient which allows for more robust experimental designs.

By using LI data and harvest index data, the efficiencies of photosynthesis and respiration can be calculated. Both of these processes can be highly variable with drought stress and are difficult to measure accurately in field conditions with adequate replication under similar conditions of light and soil moisture content. Variety response to drought conditions can be characterized by changes in one or more of the four major components in crop efficiency and is a fundamental goal of this research.

#### Weed competition

Use of herbicides in quinoa production is rare, as much of quinoa is produced organically and there are currently no herbicides labeled for quinoa. Therefore, weed control is a key issue in successful crop production. While many growers report quinoa to be exceptionally competitive with weeds, most describe a critical period early in the growing season when quinoa is highly susceptible to weed pressure (Aguilar and Jacobsen, 2003; Peterson and Murphy, 2015). Limited research on the competitive interactions between quinoa and weeds has been published to date (Jacobsen et al., 2010; Johnson and Ward 1993).

Interactions between crops and weeds are highly dependent on the density of species populations. In replicated greenhouse studies, there are generally three distinct designs to examine the interactions of weeds and crops: pair-wise, replacement or additive models (Gibson et al., 1999; Rejmánek et al., 1989; Snaydon, 1991). Pair-wise designs use a fixed ratio of two species, typically 1:1, whereas replacement and additive models take different approaches to how the species density is varied (Gibson et al., 1999). In a replacement series model, the total plant population is held constant and the ratio of crop to weed species is varied at a predictable rate while an additive model varies the total density of plants, generally holding the crop density constant and varying the weed species density (Rejmánek et al. 1989). The replacement series model ranges from exclusively crop treatments to exclusively weed treatments with intermediary ratios of species in an attempt to quantify relative competitive qualities of a crop (Rejmánek et al. 1989).

As with cultivated plant species, weed species respond to nutrient availability in a variety of ways. Some weeds are considered luxury nutrient users and increase growth proportionally to available nutrients whereas other species reach their maximum growth rates with very little nutrient inputs. Blackshaw et al. (2003, 2009) determined the response of several weed species to both N and P fertilizer inputs. Species were grouped into four response levels based on changes in biomass measurements of weeds at different fertility levels. By comparing quinoa to weed species that vary in their relative response to nutrient inputs, a replacement series design can describe the relative competitive qualities of quinoa. Additionally, by repeating the replacement series at both

high and low nutrient input levels, a wide range of potential impacts on interspecies competition can be assessed. Knowledge of the relative competitive abilities of quinoa will allow for further targeted research to achieve the goal of allowing successful large scale incorporation.

The future challenges in the Intermountain West require new management options to maintain the sustainability of farms. Incorporating a novel crop suited for drought and salinity while enhancing soil health may be an ideal approach and serve as the basis for the research presented here. This dissertation is in a multi-paper format and each chapter has been formatted according to the target journal requirements. Chapter three reports on an organic quinoa field trial of differing strip- and inter-cropping systems. Chapter four examines irrigation effects on quinoa growth with a line source field trial. Chapter five details a replacement series weed competition greenhouse study. In Chapter six, a comparison of prior cropping history and compost addition effects on soil health parameter is presented. The target journals for chapters three through six are *Agroecology and Sustainable Food Systems*, *Agricultural Water Management*, *Weed Research*, and *Applied Soil Ecology*, respectively.

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## CHAPTER III: QUINOA AS A NOVEL CROP FOR ORGANIC CROPPING SYSTEMS IN UTAH

### Abstract

Increasing crop diversity breaks pest cycles, fosters soil health and increases farm sustainability. The integration of quinoa (*Chenopodium quinoa* Willd.) as an alternative crop within the Western United States could provide a high market value crop that is tolerant to drought and marginal soils. The objective of this study was to evaluate the efficacy of different organic cropping systems for quinoa. A field trial was established as a random complete block (RCBD) with split plot and four replicates. The whole plot factor was cropping system [three levels: strip crop with hairy vetch + winter wheat mowed and blown into the crop row (SC), undersown clover (UC), and tillage only (T)]. The split plot factor was fertility [compost added (+C) or no compost added (-C)]. Steer manure compost was added prior to the first year of quinoa production at a rate sufficient to supply readily available phosphorous (P) for 2 years (11.2 Mg DM ha<sup>-1</sup>). Seed set was problematic in both years with no seed in 2013 and limited yield in 2014. Compost increased seed yield, total biomass, readily mineralizable carbon, soil respiration and microbial biomass, as measured by substrate induced respiration. In 2014, yield per row of quinoa was greater in SC than UC, with T intermediate. However, when total cropping area is accounted for, T had higher seed yields and total dry weight than UC with SC intermediate. Extractable soil nitrate (NO<sub>3</sub><sup>-</sup>) was lower in SC+C and higher in T-C than other treatment combinations, demonstrating complex interactions between readily

available nitrogen and carbon. Cropping system influence was mixed, however compost increased quinoa growth and soil microbial activity. Quinoa's suspected sensitivity to high temperatures poses a problem for widespread production in the region.

## Introduction

Organic markets have increased in size and scope in recent years. Consumers cite health, safety, and environmental concerns as top motivating factors in willingness to pay a premium price for organic foods (Oberholtzer et al., 2005). Organic diets have been shown to reduce exposure to pesticide residues (Lu et al., 2006) and organic cropping systems reduce environmental risks (Jensen and Hauggaard-Nielsen 2003). However, organic growers face steep challenges in developing cropping systems to adequately control pests and foster soil health, all while maintaining a profit. The incorporation of quinoa into organic cropping systems in the western US region could provide a novel and potentially profitable addition to crop rotations and therefore increase the sustainability of organic farms.

Quinoa is recognized as a highly nutritious substitute for traditional grains, as it provides a complete protein which is gluten free (Kozioł, 1992). Quinoa is mostly produced organically and annual imports into the US exceed 23,000 tons (FAO 2013). While over 90 % of the US market is imported, the diverse ecosystems in which it is grown in South America indicate there may be varieties that could fit well into regional growing conditions of the western US (Jacobsen et al. 2003). Quinoa prices have increased 124% between 2011 and 2014 and the US is the largest importer (USDA FAS 2014). Growers in South America have been under great pressure to increase production

to meet the growing demand, which has intensified farming practices and reduced sustainability (Jacobsen, 2011).

In order to determine whether quinoa could be a viable alternative crop for the western US, appropriate cropping systems need to be developed. Quinoa has been traditionally grown without synthetic fertilizers, only manure inputs; however, quinoa has been shown to respond to nitrogen (N) rates of up to 120 kg/ha (Aguilar and Jacobsen, 2003; Schulte auf'm Erley et al., 2005). Crop nutrient requirements in organic systems are frequently met with a combination of cover crops, diverse rotations, and applications of compost or manure. Compost use increases soil organic matter (SOM), which is frequently low in the western arid soils and can increase soil quality indicators such as plant available nutrients and water holding capacity (Reeve et al. 2012; Olsen et al. 2015). However, the application of compost can be cost prohibitive and may cause elevated levels of other nutrients, particularly phosphorous (P), which may become an environmental concern. Alternatively, some growers rely on cover crops or intercropping alone to address soil fertility. Using a nitrogen fixing green manure crop as a cover, relay, or intercrop can provide significant inputs of N while also suppressing weeds and others pests (Altieri 1999; Altieri and Letourneau 1982; Wang et al. 2011).

Each of these systems has benefits and drawbacks, which must be considered carefully based upon crop, pest, and field conditions. Cover crops increase SOM and while they do not contribute other nutrients to the system, such as P and potassium (K), cover crops can help recycle nutrients from deeper layers in the soil (Cherr et al. 2006; Dabney et al. 2001). Intercropping has been shown to increase the productivity of a



cropping system by increasing plant-available nutrients. The impact of these interactions have been reported as increased plant growth indicators such as tissue nutrient content and yield (Gao et al., 2009; Li et al., 2001). However, these interactions can be species dependent. An intercropping approach does not always benefit the main crop due to the potential for competition, which can reduce crop yield, and impact N uptake and water use efficiency in some systems (Gao et al., 2009; Zhang et al., 2008, 2007). Therefore, crop selection and timing of establishment and termination of growth must be well developed. In colder climates, with short growing seasons, establishing cover crops and allowing for sufficient growth to serve as a green manure can be challenging. Strip cropping with a green manure, relay cropping, or intercropping may extend the growing season and increase nutrient contribution, thereby offsetting any interspecific competitive effects.

The goal of this research was to measure the growth response of quinoa in three organic cropping systems designed to supply nitrogen through various configurations of cover crops with and without added compost: Cover crop systems tested were: (1) a winter cover crop of 70% hairy vetch (*Vicia villosa* Roth.) and 30% winter wheat (*Triticum aestivum* L.) incorporated with tillage prior to seeding quinoa (tillage-only, T); (2) the same hairy vetch and winter wheat cover crop followed undersowing with clover once the quinoa is established (undersown clover, UC); and (3) a cover crop of hairy vetch and winter wheat strip cropped with quinoa where mowed residue of the cover crop is blown onto the quinoa row (strip crop, SC). Quinoa growth and yield were determined as well as soil chemical, biological, and physical properties, to evaluate the impact of

cropping system and compost additions on quinoa production and soil quality.

Hypotheses tested were (1) quinoa grown with compost in an intercropped system will have greater growth and yield than in a tillage-only system and (2) intercropping will increase soil quality indicators when compared to tillage-only treatments.

## Materials and methods

### *Field design and management*

The experimental site was located on the Utah State Greenville Experiment Station Organic Research Farm in North Logan, UT. The soil was a silt loam (Millville silt loam, USDA Web Soil Survey). The site had been managed organically since 2005 with a variety of summer and winter cover crops with no additional inputs, and certified organic in 2011. Field corn was grown in 2010 and pumpkins in 2011. The experimental design was a random complete block (RCBD) with split plot and four replicates, for a total of 24 plots. The whole plot factor was cropping system [three levels: strip crop mow and blow (SC), under-sown clover (UC) and tillage only (T)] and the split plot factor was fertility [compost added (+C) or no compost added (-C)].

On August 29<sup>th</sup> 2012, the field was planted with a cover crop consisting of a hairy vetch/winter wheat mix (78/34 kg ha<sup>-1</sup>, respectively) prior to establishment of all plots and treatments in the spring of 2013. On May 17<sup>th</sup> 2013 the winter cover crop mix was tilled-in prior to seeding quinoa, with the exception of 122 cm wide strips within the SC treatments. Steer manure compost was applied in April 2013 prior to planting the first crop of quinoa at a rate sufficient to supply readily available phosphorous (P) for 2 years. Using published yield response data for P in quinoa, this was calculated as 11.2 metric

tons ha<sup>-1</sup> (dry weight) of composted steer manure (Table 1). Quinoa rows were spaced 46 cm apart with a seeding rate of 13.4 kg ha<sup>-1</sup>. Quinoa variety Oro de Valle (Washington State University) was planted on June 4, 2013. The remaining strips of cover crops (SC plots) were mowed and the residue raked onto the quinoa rows after quinoa emergence to simulate mowing with a side discharge mower. Overhead sprinkler irrigation was used from June through harvest applying approximately 5-8 cm of water per week, depending on weather conditions. Weeds were controlled between rows with hoeing and in-rows by hand roguing twice per season within 60 days of seeding. The clover in the UC plots was broadcast seeded (13.4 kg ha<sup>-1</sup>) and lightly incorporated with a rake once the quinoa was well established (approximately late June) to avoid excessive competition. After harvest on September 3, 2013, the clover remained as the overwintering cover crop in the UC plots while a second hairy vetch winter wheat cover crop was planted in the SC and T treatments. All winter cover crops were incorporated approximately 1 wk prior to spring planting in April 2014 as described above. The variety Oro de Valle was planted on April 25, 2014. Poor germination required a second, shallow tillage and re-seeding on May 28<sup>th</sup> with the variety Cherry Vanilla due to a shortage of Oro de Valle with desired germination rates. In the SC treatment the location of strips of quinoa and cover crop within each plot were switched.

#### *Plant analyses*

Quinoa plants were sampled mid-season (July 10, 2013 and June 20, 2014). Ten plants per plot were cut at ground level from the center of each plot, weighed, dried at 60°C, weighed dry and then analyzed for total N by combustion according to the

manufacturer's protocol (Skalar Primac Total Nitrogen Analyzer, Skalar Primac SLC Carbon Analyzer, respectively, Salt Lake City, Utah). A single row of 3 m length within the center of each plot was selected for harvest (September 3, 2013 and September 16, 2014). Any weeds in the quinoa row were also harvested to assess weed pressure. In 2013 the quinoa did not set any seed, likely due to high air temperatures encountered during flowering (Murphy and Matanguihan, 2015). Due to the absence of grain in 2013, plants within this section were removed and sectioned into root, stem and panicles. Plant portions were weighed wet, then dried, re-weighed and analyzed for N as described above. Cover crops were sampled for biomass before each mowing or incorporation with tillage. A 0.46 x 3.05 m sample from the center of each plot was cut at ground level. Individual species were separated, weighed wet, and then dried and processed as described above for total N content.

The percentage of ground cover occupied by weeds was determined at harvest of each year visually at three random locations between the quinoa rows using a 1/10 m area to assess weed pressure. Similarly, the percentage of ground cover by clover plants was determined at three random locations between the quinoa rows.

#### *Soil analyses*

##### *Soil chemical properties*

Soils were sampled twice per growing season (July 9 and September 30, 2013; July 2 and September 2, 2014), corresponding to 30 days of crop growth and crop dry down. Six soil subsamples per plot were collected from 0 to 30 cm using a 2.5 cm corer and combined in the field. Soils were sieved through a 4 mm screen, stored in re-sealable

plastic bags and refrigerated at 4°C until processing within 10 days. Nitrate and ammonium-N ( $\text{NH}_4^+$ -N) were extracted in 1M KCl, and analyzed by Lachat (Quickchem 8500, Hach Company, Loveland, CO) using sulfanilamide and phenate methods, respectively according to manufacturer protocols. Soil EC and pH was measured in a 1:2 soil:water suspension once per season. Soil P and K levels were measured in samples collected in July using the Olsen method (Gavlak et al., 2003). Total N and total C were measured by combustion from air-dried soils collected in July according to the manufacturer's protocol (Skalar Primacs Total Nitrogen Analyzer, Skalar Primac SLC Carbon Analyzer, respectively, Salt Lake City, Utah). Particulate organic matter was measured on soils collected prior to harvest in fall 2014 following Cambardella and Elliot (1992).

#### *Soil biological properties*

To assess microbial characteristics, soils were sampled and stored at 0 to 10 cm on the same dates listed above in section 2.3.1. Soil  $\beta$ -glucosaminidase activity was determined using 2.5 g oven-dry weight equivalent (od eq) soil at 22% moisture according Parham and Deng, (2000). The resulting color intensity was measured using a microplate reader (Spectramax M2, Molecular Devices, Sunnyvale, CA). Mineralizable carbon (minC), soil basal respiration (BR) and active microbial biomass (MB) was measured on the same soils according to Anderson and Domsch (1978). Sealed vials containing 5 g od eq soil at 22% moisture content were incubated at 25°C. Carbon dioxide ( $\text{CO}_2$ ) measured in the headspace after 11 days was considered minC. Vials were uncapped, flushed for one minute using moisture saturated air, and then recapped and the

hourly rate of CO<sub>2</sub> production measured for BR after exactly 2 hrs. Active MB was measured on the same samples by adding 0.5 mL of 60 g L<sup>-1</sup> aqueous solution of glucose, resting the samples for 1 hour uncapped, recapping the vials for 2 hours, and then removing 2 mL of air from headspace CO<sub>2</sub> with a syringe for analysis. An infrared gas analyzer (model 6251, LICOR Biosciences, Lincoln, NE) was used to measure CO<sub>2</sub> in the headspace. All samples per analysis were started on the same day within 10 d of sampling, conducted on moist soil, and measured in triplicate.

#### *Soil physical properties*

Aggregate stability was determined with a wet sieving apparatus (Eijkelkamp, Giesbeek, NL) on soils collected in September 2, 2014. Samples were air-dried, with care taken to ensure soil aggregates remained intact. The manufacturer's protocol was followed to provide a ratio of the weight of stable aggregates to total aggregates. Also in the fall of 2014 prior to tillage, soil bulk density was determined using a truck mounted 4 cm diameter Giddings soil probe. Sections were sampled from 0 to 45 cm in depth from which cores were sectioned into 5 cm depths. An intact subsection with length of 4 cm within each depth range was transferred into a tin, weighed wet and then dried at 105°C for a minimum of 24 hours, or until the decrease in weight due to moisture loss had stopped. Soil bulk density ( $\rho_b$ ) was calculated using the equation:

$$\rho_b = \frac{\text{oven dry weight of soil (g)}}{\text{volume of soil (cm}^3\text{)}}$$

### *Statistical analyses*

Cropping system and compost treatment comprised a two-way factorial in a RCBD mixed model where plot was the experimental unit and month and year repeated measures. A mean was computed at the plot level for all subsamples. The response variables of quinoa biomass and yield and soil chemical, biological, and physical measures were assessed using analyses of variance with PROC GLIMMIX in the Statistical Analysis System for Windows version 9.4 (SAS Institute, Cary, NC). Variables were square-root or log transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. Multiple means comparisons were adjusted using the Tukey method to control for family-wise Type I error rate.

### Results

#### *Quinoa growth and yield*

In 2013, no seeds were produced due to peak summer temperatures coinciding with the period of flowering and seed set. In 2014, seed yields were greater ( $p=0.02$ ) in the SC than UC treatment with T intermediate, while quinoa grown with compost had greater ( $p<0.01$ ) seed yield than without (Figure 1 A and B). However, when total cropping area was accounted for using a land equivalence ratio (LER) of 0.45, yields in the T treatment were greater than UC with SC intermediate ( $p=0.03$ ). The impact of compost on yield remained the same ( $p=0.01$ ) (Figure 1 C and D).

In order to capture treatment effects on quinoa growth in lieu of seed yield, biomass partitioning data for both years was collected as panicle, stem, and total above ground biomass. There were two interactions that define the limited effects of cropping

system on biomass (Table 3). First, an overwhelming effect of year and/or variety on crop growth was observed, rather than differences due to cropping system as evident in the year\*cropping system interaction for total biomass ( $p=0.02$ ). Within each year, total biomass was similar between cropping system; however, when compared between years, quinoa in the SC system in 2013 had much less biomass than T or SC in 2014 ( $p=0.03$  and  $p=0.01$ , respectively) (Figure 2 and Table 3). There was also a significant cropping system by compost interaction ( $p=0.02$ ) in stem dry weight (Table 4). Within each cropping system, quinoa receiving compost had greater total biomass than without, with no differences between cropping systems noted ( $p<0.01$  for all comparison except UC+C versus T-C  $p=0.04$ ).

Compost application had an impact on all biomass measures. The interaction of compost and year was significant for panicle, stem, and total dry weight at harvest ( $p<0.001$ ,  $p<0.01$  and  $p=0.04$ , respectively). Regardless of year, quinoa with compost had higher stem weight and total dry weight than without compost ( $p<0.01$ ). The Cherry Vanilla variety with compost had greater total biomass, stem weight, and panicle weight than the Oro de Valle variety ( $p<0.01$ ) planted in 2013. Quinoa with compost had greater panicle weights in 2014 than without compost; but no differences in panicle weights were observed in 2013 regardless of compost level (Figure 3, Table 4). Stems and total biomass were lightest when quinoa did not receive compost, regardless of year and heaviest in 2014+C while 2013 +C was intermediate ( $p<0.01$  for all comparisons). When LER was applied to total quinoa biomass, panicle, and stem weights, the tillage (T) system had greater biomass than SC ( $p=0.02$ ), and the UC treatment was intermediate.



There were no differences in tissue N at sampling mid-season in either year (data not shown).

Weed pressure within plots was also impacted by cropping system and compost. At harvest, there was significantly greater weed biomass in 2013 than 2014 ( $p < 0.01$ ), regardless of cropping system or compost level (Table 5). Percent ground coverage by weeds and or clover at the end of each growing season was significantly greater for UC than both T and SC, which did not differ from each other ( $p < 0.01$ , Figure 4). The interaction between compost and year on percentage of weed ground cover was also significant ( $p < 0.01$ ) (Figure 5 and Table 5). The effects of compost were more pronounced in 2014 than 2013. Plots without compost in 2014 had more ground cover than with compost ( $p < 0.01$ ), yet there was no difference in ground cover between compost levels in 2013 ( $p < 0.01$  for all comparisons). Plots without compost in 2014 also had greater weed coverage than 2013+C ( $p < 0.01$ ).

#### *Soil chemical properties*

Both compost and cropping system impacted soil properties, although compost had the dominant effect. Soil total organic carbon (TOC) was affected by the interaction of compost and year ( $p = 0.02$ ). Similar to quinoa biomass data, TOC was greater ( $p < 0.01$ ) in the compost treatment in 2014 than in than in any other compost/year combination (Figure 6), perhaps due to higher biomass returns. Cropping system and compost interactions were also significant for TOC. Under-sown clover with compost (UC+C) had higher TOC ( $p < 0.01$ ) than UC-C with all other cropping system/compost combinations

intermediate (Figure 7). There were no significant differences between treatments in amount of total soil N (data not shown).

Soils that did not receive compost had higher soil  $\text{NO}_3^-$  at the end of the growing season than those with compost, likely as a result of poor quinoa growth and ability to assimilate available  $\text{NO}_3^-$ . This was evident in two interactions. First, the interaction between cropping system, compost and month was significant ( $p=0.02$ ) for soil extractable  $\text{NO}_3^-$  (Figure 8). There were no differences in soil  $\text{NO}_3^-$  between cropping system/compost combinations in July. However, in September, T-C had more  $\text{NO}_3^-$  than all treatment combinations except SC-C ( $p<0.001$  for all comparisons); there was also more soil  $\text{NO}_3^-$  in SC-C than UC-C in September ( $p=0.05$ ) (Figure 8). Secondly, year also interacted with month, compost, and soil nitrate levels ( $p<0.001$ ). There was no difference between  $\text{NO}_3^-$  levels regardless of compost level in July; however, soil  $\text{NO}_3^-$  was higher in -C than +C in September 2014 ( $p=0.038$ ), suggesting that quinoa with compost removed more  $\text{NO}_3^-$  than without that year (Figure 9).

There was a significant year by month interaction in amount of extractable soil  $\text{NH}_4^+$  ( $p<0.01$ ). Soils in July 2014 had higher  $\text{NH}_4^+$  than any other year month combination ( $p<0.01$ , data not shown) possibly due to favorable temperatures and moisture for microbial activity. Overall low levels of soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  suggest tight coupling between mineralization and nitrification processes and quinoa uptake. Available soil P was low overall but significantly greater in plots with compost than without ( $p<0.01$ , Figure 10). A positive correlation ( $p=0.029$ ) between Olsen P and panicle

weights was observed (Table 6). Soil  $\text{NO}_3^-$  levels in July were positively correlated with panicle, stem, and total biomass ( $p=0.001$ ,  $0.004$  and  $0.001$ , respectively). Compost levels were positively correlated with P and negatively correlated with soil  $\text{NO}_3^-$  in September ( $p=0.011$  and  $0.008$ , respectively), which may indicate greater N uptake by amended quinoa.

#### *Soil biological properties*

Soil biological activity was also affected by cropping system, compost level and sampling date. For readily mineralizable carbon (RMC), the interaction of compost, year and month was significant at  $p=0.009$ . RMC was higher in 2014 than 2013, and higher in composted plots than plots without compost in July 2013 and September 2014, perhaps due to differences in quinoa variety or high variability in field conditions combined with a lack of statistical power ( $p<0.0001$  for all comparisons) (Figure 11 and Table 7). The interaction of compost and year was also significant for microbial biomass ( $p=0.036$ ). Soils in 2014 had greater MB than in 2013 within compost level and MB was greater in soils with compost than without in 2014 only ( $p<0.001$ ) (Figure 12). There was no difference in MB between cropping systems within each year; however, there was a significant interaction of cropping system and year ( $p=0.043$ ) (Figure 12). Similar to quinoa biomass and TOC, both UC and SC cropping systems had higher MB in 2014 than 2013 which may indicate a positive trend in enhancing soil health over time with intercropped or relay-cropped systems ( $p<0.01$  for all comparisons except SC2014 vs SC2013  $p=0.048$ ).

Conversely, basal respiration (BR) was greater in 2013 than 2014 in both July and September, regardless of compost level (Figure 13). Basal respiration was higher in composted treatments in July 2013 ( $p < 0.001$ ) and September 2014 ( $p < 0.001$ ) but there were no difference in September 2013 or July 2014.

There were no treatment effects observed for  $\beta$ -glucosaminidase, only the interaction of year and month was significant at  $p = 0.0005$  (Figure 14). September 2013 was greater than July 2013 and September 2014, while July 2014 was intermediate ( $p = 0.010$  and  $p = 0.005$ , respectively).

#### *Soil physical properties*

Bulk density sampled between 0 and 15 cm was affected by the interaction of cropping system and compost ( $p < 0.01$ ). While likely not of consequence to crop production, bulk density was greater in UC+C plots than T+C, SC-C and SC+C ( $p < 0.01$ ,  $p < 0.01$ , and  $p = 0.01$ , respectively) with UC-C and T-C intermediate (Figure 15). Neither bulk density nor aggregate stability at the lower depth (15 to 30 cm) were impacted by any combination of cropping system or compost.

#### Discussion

Large-scale growers tend to avoid using compost as it is costly to transport and apply. However, in our study, the application of compost enhanced both seed and biomass yields, lowered weed pressure, and increased the soil fertility/health indicators of soil P, TOC, and microbial biomass. Plants that received compost produced more seed, were larger and crop canopies closed faster, which reduced weed pressure. In this study,

quinoa without compost had limited response to nitrogen additions from cover crops, only evident in the seed yields per linear row of quinoa in SC plots. Available soil N is more mobile than P, and is likely to be assimilated as rapidly as it is mineralized in a low N system such as our study, which may explain the lack of nitrate differences observed. Instead, the increased growth with compost is likely due to significant increase in available P or a combination of both. Although researchers in Colorado observed no response to P additions, other recommendations range from 30 kg P ha<sup>-1</sup> to 80 kg P ha<sup>-1</sup> (Aguilar and Jacobsen, 2003; Darwinkel and Stolen, 1997; Murphy and Matanguihan, 2015; Oelke et al., 1992). Soil type greatly affects the availability of soil P as demonstrated in this study and by Bai et al (2013) who observed critical Olsen P levels for yield of rice, maize and soybean systems ranging between 10.9 to 21.4 mg kg. At our site, soils without compost had available P levels of 3 mg kg soil, far below the recommended value of 15 mg kg<sup>-1</sup> for most crops in our region (James and Topper, 1993). No differences were noted in P availability between cropping systems as have been reported in some intercropped systems (Betencourt et al., 2012; Li et al., 2008, 2001). This suggests P availability, as the limiting nutrient, was only relieved by the addition of compost, allowing for quinoa to respond to varied N levels.

In addition to compost, quinoa growth was greatly affected by weather. Quinoa flowered in July and August in both years, which coincided with peak summer temperatures. Between July 1<sup>st</sup> and August 31<sup>st</sup>, 2013 the average high temperature was 32.2°C. Varieties currently available in North America are thought to be susceptible to pollen sterility or seed abort above 32°C (Murphy and Matanguihan, 2015). In order to

escape peak temperatures during flowering, seeding was moved earlier to April in 2014; however, poor germination rates that necessitated re-seeding resulted in a similar timeline as in 2013. Coincidentally, the late summer period in 2014 was cooler, with an average high temperature of 30 °C allowing for seed development. Tolerance to high summer temperatures is currently a critical limiting factor to the successful adoption of quinoa in Utah and the Western region.

Despite the lack of seed set in 2013, biomass data and soil health indicators provide strong support for the benefits of compost and diverse cropping systems. Short growing seasons frequently limit spring cover crop growth prior to establishing a cash crop and only leave a small window to establish a fall cover crop after a late harvested cash crop (Cherr et al., 2006). However, the benefits of incorporating cover crops as green manure have been proven even in short growing seasons (Griffin et al., 2000; Cherr et al., 2006). Treatment combinations in this study provided sufficient N for growth in 2013, but the later seeding combined with earlier termination of the cover crop in 2014, provided little N input for the UC-C and T-C treatments in 2014. Carryover N from cover crops and compost proved sufficient for quinoa growth in 2014 but could become deficient in the long term unless quinoa is rotated with a shorter season cash crop to provide a greater window for cover crop growth.

Strip- or inter-cropped systems have been used to overlap with the cash crop in time and space to minimize the effects of short growing season and maximize land use. The differences between the total amounts of N incorporated through the different cover crop systems in this study are evident (Table 2). Competitive interactions in strip crop

and intercrop systems often lower yields, however. Seed weights in 2014 were much lower in the UC system, perhaps due to excessive competition between clover and quinoa. In this instance, the minimal additional N inputs provided by clover may not outweigh the reduction in yield due to competition. On a harvested row basis, the SC treatment had the highest seed weights as well as the highest N inputs and may have also benefitted from reduced competition through additional separation between the cover crop and quinoa. The spatial arrangement and size ratios of intercropped species are critical in determining the yield of both species, and hence the feasibility of the system (Chen et al., 2004; Zhang et al., 2007). As a result of the system design, the SC system had a much larger footprint, therefore reducing the yield per Ha. In fact, in our study, the ratio of quinoa to total cropping system area was 0.45, which would reduce the effective seed yield of quinoa to  $4.18 \text{ kg ha}^{-1}$ , intermediary between T and UC ( $6.19$  and  $1.25 \text{ kg ha}^{-1}$ , respectively). Total yield potential is, of course, important when choosing an organic cropping system; however, the benefits to soil fertility and health and hence long term farm sustainability may be greater in an inter- or strip-cropped system over tillage alone.

Although this study covered only two growing seasons, MB and TOC increased in the inter- and strip-cropped systems while no change was observed in the tillage only system. This may be indicative of cropping system effects that would become more evident over time. Higashi et al. (2014) found cover crops instead of bare fallow and reduced tillage increased soil organic carbon over the course of 2-9 years. When combined with cover crops, the no-tillage treatments had the highest SOC; but treatments with a rotary tiller still increased SOC while moldboard plow tillage saw no increase. In

long-term residue and tillage management trials, B-glucosaminidase has been closely correlated with organic C in the upper soils layer after mulch treatments as a sensitive indicator of N mineralization (Ekenler and Tabatabai 2002; Ekenler and Tabatabai 2003). However, the authors report significant decreases in B-glucosaminidase activity as depth increases, with levels nearly half as great deeper than 5 cm (Ekenler and Tabatabai 2003). Soils sampled in our study were homogenized from 0-15 cm, which could have obscured any near surface increases in the SC system. Since the levels of available N did not show difference as samples dates either, the timing of sampling may not have been coincident with differences in mineralization rates. Instead, B-glucosaminidase testing accomplished closer to the surface and timed near the incorporation of cover crops may provide more useful insight.

By far, the addition of compost had the greatest impact on plant and soil health in this study and has the potential to provide long-term carryover benefits. The long-term effects of a one-time compost application have been reported to benefit soil fertility and health indicators for at least 3-4 years (Olsen et al. 2015; Eghball et al., 2004). Growers weighing the costs and benefits of compost application need to understand and account for the long-term benefits as well as the potential for synergistic effects between compost and cover crops such as nutrient cycling and moisture availability. Our results confirm that compost cover crop combinations build soil carbon, soil fertility, and increase microbial biomass; however, more time is likely needed to differentiate potential soil impacts of the different cover cropping systems.



## Conclusion

Quinoa may provide a beneficial novel crop for Utah and the Western region; however, the wide scale adoption of quinoa is not feasible until the development of varieties tolerant to summer temperatures in excess of 32 °C during flowering and seed set are available. Quinoa growth was increased with the addition of compost, likely due to a direct response to available P, as response to N was limited in non-compost plots. Strip cropped systems had highest seed yield per row; however, when equivalent land areas are accounted for, the systems were not different in seed yield highlighting the importance of compost addition in a low P system. Compost application increased readily mineralizable soil carbon and microbial biomass. Unlike tillage, the relay- and inter-cropped systems showed increases in soil microbial biomass over time.

Although we saw differences among cropping systems and compost treatment combinations, the time frame of this study was insufficient to capture the full extent of soil health implications of inter- and strip-crop systems. Ideally, a longer-term study would be constructed to follow the systems through several seasons with adequate crop rotation to increase cover crop growth and allow sufficient time for greater changes in the soil ecosystem. The interaction between inter- or strip-cropped plants and the effects on long-term nutrient availability to the quinoa crop cannot be fully determined in a shorter term study.

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## Tables and figures

Table 1. Composted steer manure nutrient analysis (analysis on air-dried compost).

| Parameters    | Value |
|---------------|-------|
| Moisture %    | 4.9   |
| pH (2:1)      | 8     |
| EC (2:1) dS/m | 4.32  |
| N %           | 1.54  |
| C %           | 24.5  |
| P %           | 0.6   |
| K %           | 1.32  |
| Ca %          | 3.72  |
| Mg %          | 0.74  |
| S %           | 0.33  |
| Na mg/kg      | 3410  |
| B mg/kg       | 17.3  |
| Zn mg/kg      | 212   |
| Cu mg/kg      | 31.2  |
| Fe mg/kg      | 5980  |
| Mn mg/kg      | 254   |

Table 2. Total nitrogen inputs based on above ground biomass by cropping system averaged over blocks. Cover crop and clover residues were incorporated with tillage prior to quinoa seeding while compost was raked in following tillage in 2013.

| Input                               | Under-sown<br>clover        | Tillage                     | Strip crop                  |             |
|-------------------------------------|-----------------------------|-----------------------------|-----------------------------|-------------|
|                                     | kg total N ha <sup>-1</sup> | kg total N ha <sup>-1</sup> | kg total N ha <sup>-1</sup> |             |
| <b>Cover crop<sup>1</sup></b>       |                             |                             |                             |             |
| 2013                                | 62.8                        | 62.8                        | 62.8                        |             |
| 2014                                | -                           | 7.87                        | 7.87                        |             |
| <b>Clover<sup>2</sup></b>           |                             |                             |                             |             |
| 2013                                | -                           | -                           | -                           |             |
| 2014                                | 18.2                        | -                           | -                           |             |
| <b>Compost<sup>3</sup></b>          |                             |                             |                             |             |
| 2013                                | 173                         | 173                         | 173                         |             |
| 2014                                | -                           | -                           | -                           |             |
| <b>Mow and<br/>blow<sup>4</sup></b> |                             |                             |                             |             |
| 2013                                | -                           | -                           | 195                         |             |
| 2014                                | -                           | -                           | 158                         |             |
| <b>Total</b>                        |                             |                             | <b>2013</b>                 | <b>2014</b> |
| +C                                  | 254                         | 243                         | 438                         | 401         |
| -C                                  | 81.0                        | 70.7                        | 265                         | 229         |

<sup>1</sup> Mix of 70% hairy vetch and 30% wheat

<sup>2</sup> Planted under established quinoa in 2013, over-wintered in place of cover crop mix, and tilled-in spring of 2014 as with cover crop.

<sup>3</sup> Rate of 11.2 metric tons ha<sup>-1</sup> applied only to compost treatment plots (+C).

<sup>4</sup> Two cuttings per season were raked on top of soil around quinoa rows.

Table 3. Means (n=4) for quinoa total biomass and height at harvest significant interactions. Means are adjusted and non-adjusted for land equivalent ratio of cropping systems (LER and Non-LER, respectively). All statistics are presented when treatment effects are significant ( $p < 0.05$ ).

| <b>Effect</b>                      | <b>Total biomass<br/>Non-LER<br/>Mg ha<sup>-1</sup></b> | <b>Height<br/>(cm)</b> | <b>Total biomass<br/>LER<br/>kg ha<sup>-1</sup></b> |
|------------------------------------|---|------------------------|---|
| <b><i>Year*cropping system</i></b> |   |                        |   |
| 2013 UC                            | 1.67 <sup>AB</sup>                                      | 83.2                   | 1670 <sup>AB</sup>                                  |
| 2013 T                             | 2.63 <sup>AB</sup>                                      | 91.3                   | 2630 <sup>A</sup>                                   |
| 2013 SC                            | 1.09 <sup>B</sup>                                       | 76.1                   | 490 <sup>B</sup>                                    |
| 2014 UC                            | 2.85 <sup>AB</sup>                                      | 67.8                   | 2850 <sup>AB</sup>                                  |
| 2014 T                             | 3.69 <sup>A</sup>                                       | 78.8                   | 3690 <sup>A</sup>                                   |
| 2014 SC                            | 2.77 <sup>A</sup>                                       | 64.6                   | 1250 <sup>A</sup>                                   |
| <b><i>Year*compost</i></b>         |   |                        |   |
| 2013 +C                            | 2.55 <sup>B</sup>                                       | 94.8 <sup>A</sup>      | 2250 <sup>B</sup>                                   |
| 2013 -C                            | 1.04 <sup>C</sup>                                       | 72.3 <sup>B</sup>      | 941 <sup>C</sup>                                    |
| 2014 +C                            | 4.84 <sup>A</sup>                                       | 86.7 <sup>AB</sup>     | 4090 <sup>A</sup>                                   |
| 2014 -C                            | 1.37 <sup>C</sup>                                       | 54.1 <sup>C</sup>      | 1100 <sup>C</sup>                                   |
| <b><i>ANOVA p values</i></b>       |   |                        |   |
| Year (Y)                           | 0.001   | 0.001                  | 0.001   |
| Cropping system (S)                | 0.119   | 0.147                  | 0.018   |
| Compost (C)                        | <0.001  | 0.001                  | <0.001  |
| Y*S                                | 0.015   | 0.390                  | 0.015   |
| Y*C                                | 0.042   | 0.031                  | 0.042   |
| S*C                                | 0.062   | 0.102                  | 0.062   |

Table 4. Means (n=4) for quinoa panicle and stem biomass significant interactions. Means are adjusted and non-adjusted for land equivalent ratio of cropping systems (LER and Non-LER, respectively). All statistics are presented when treatment effects are significant ( $p < 0.05$ ).

| <b>Effect</b>                      | <b>Panicle<br/>Non-LER<br/>kg ha<sup>-1</sup></b> | <b>Stem<br/>Non-LER<br/>kg ha<sup>-1</sup></b> | <b>Panicle<br/>LER<br/>kg ha<sup>-1</sup></b> | <b>Stem<br/>LER<br/>kg ha<sup>-1</sup></b> |
|------------------------------------|---|--|---|--|
| <i>Year*compost</i>                |   |  |   |  |
| 2013 +C                            | 599 <sup>B</sup>                                  | 1950 <sup>B</sup>                              | 541 <sup>B</sup>                              | 1710 <sup>B</sup>                          |
| 2013 -C                            | 213 <sup>B</sup>                                  | 827 <sup>C</sup>                               | 208 <sup>B</sup>                              | 733 <sup>C</sup>                           |
| 2014 +C                            | 2040 <sup>A</sup>                                 | 2800 <sup>A</sup>                              | 1690 <sup>A</sup>                             | 2400 <sup>A</sup>                          |
| 2014 -C                            | 617 <sup>B</sup>                                  | 753 <sup>C</sup>                               | 488 <sup>B</sup>                              | 617 <sup>C</sup>                           |
| <i>Cropping<br/>system*compost</i> |   |  |   |  |
| UC-C                               | N/A   | 489 <sup>BC</sup>                              | 243 <sup>B</sup>                              | 489 <sup>B</sup>                           |
| UC+C                               | N/A   | 2630 <sup>A</sup>                              | 1160 <sup>A</sup>                             | 2630 <sup>A</sup>                          |
| T-C                                | N/A   | 1250 <sup>BC</sup>                             | 635 <sup>B</sup>                              | 1250 <sup>B</sup>                          |
| T+C                                | N/A   | 2740 <sup>A</sup>                              | 1690 <sup>A</sup>                             | 2740 <sup>A</sup>                          |
| SC-C                               | N/A   | 630 <sup>C</sup>                               | 166 <sup>B</sup>                              | 284 <sup>B</sup>                           |
| SC+C                               | N/A   | 1760 <sup>AB</sup>                             | 497 <sup>B</sup>                              | 791 <sup>B</sup>                           |
| <i>ANOVA p values</i>              |   |  |   |  |
| Year (Y)                           | <0.001  | 0.006  | <0.001  | 0.024                                      |
| Cropping system (S)                | 0.066   | 0.129  | 0.008   | 0.018                                      |
| Compost (C)                        | <0.001  | <0.001   | <0.001  | <0.001                                     |
| Y*S                                | 0.326   | 0.268  | 0.312   | 0.334                                      |
| Y*C                                | 0.001   | 0.002  | 0.002   | 0.003                                      |
| S*C                                | 0.176   | 0.024  | 0.002   | 0.001                                      |



Table 5. Means (n=4) for weed biomass significant main effect of year. Means are both adjusted and non-adjusted for land equivalent ratio (LER and Non-LER, respectively) of cropping systems. All statistics are presented when treatment effects are significant ( $p < 0.05$ ).

| <b>Effect</b>         | <b>Weed biomass<br/>Non-LER<br/>kg ha<sup>-1</sup></b> | <b>Weed biomass<br/>LER<br/>kg ha<sup>-1</sup></b> |
|-----------------------|--|--|
| <i>Year</i>           |  |  |
| 2013                  | 871 <sup>A</sup>                                       | 691 <sup>A</sup>                                   |
| 2014                  | 258 <sup>B</sup>                                       | 231 <sup>B</sup>                                   |
| <i>ANOVA p values</i> |  |  |
| Year (Y)              | 0.001  | 0.001  |
| Cropping system (S)   | 0.549  | 0.191  |
| Compost (C)           | 0.523  | 0.945  |
| Y*C                   | 0.300  | 0.343  |

Table 6. Pearson's correlation coefficients and p-values between available soil P and NO<sub>3</sub><sup>-</sup> levels and compost applications, quinoa biomass, and seed yield averaged over year.

|                      | <b>P</b>     | <b>NO<sub>3</sub><sup>-</sup><br/>July</b> | <b>NO<sub>3</sub><sup>-</sup><br/>September</b> |
|----------------------|--------------|--|---|
| <b>Compost</b>       | <b>0.368</b> | <b>-0.059</b>                              | <b>-0.379</b>                                   |
| p-value              | 0.011        | 0.692                                      | 0.0079  |
| <b>Panicle</b>       | <b>0.319</b> | <b>0.535</b>                               | <b>-0.209</b>                                   |
| p-value              | 0.029        | 0.0001                                     | 0.154   |
| <b>Stem</b>          | <b>0.199</b> | <b>0.4097</b>                              | <b>-0.253</b>                                   |
| p-value              | 0.1790       | 0.004                                      | 0.0829  |
| <b>Total biomass</b> | <b>0.258</b> | <b>0.479</b>                               | <b>-0.244</b>                                   |
| p-value              | 0.080        | 0.001                                      | 0.094   |
| <b>Seeds</b>         | <b>0.235</b> | <b>-0.030</b>                              | <b>-0.258</b>                                   |
| p-value              | 0.284        | 0.889                                      | 0.224   |

Table 7. Means (n=4) for readily mineralizable C, soil respiration and microbial biomass interactions of year and month. All statistics are presented when treatment effects are significant ( $p < 0.05$ ).

| Effect                | Readily mineralizable C<br>mg kg soil <sup>-1</sup> |                   | Soil respiration<br>mg kg soil <sup>-1</sup> hour <sup>-1</sup> |                   | Microbial biomass<br>mg kg soil <sup>-1</sup> |                  |
|-----------------------|---|-------------------|---|-------------------|---|------------------|
|                       | July  | September         | July  | September         | July  | September        |
| <i>Year*month</i>     |   |                   |   |                   |   |                  |
| 2013                  | 11.1 <sup>D</sup>                                   | 15.1 <sup>C</sup> | 3.75 <sup>A</sup>   | 3.61 <sup>A</sup> | 377 <sup>A</sup>                              | 267 <sup>C</sup> |
| 2014                  | 23.0 <sup>B</sup>                                   | 29.0 <sup>A</sup> | 2.03 <sup>B</sup>   | 1.75 <sup>C</sup> | 352 <sup>B</sup>                              | 401 <sup>A</sup> |
| <i>ANOVA p values</i> |   |                   |   |                   |   |                  |
| Year*month            | 0.009   |                   | 0.037   |                   | <0.001  |                  |

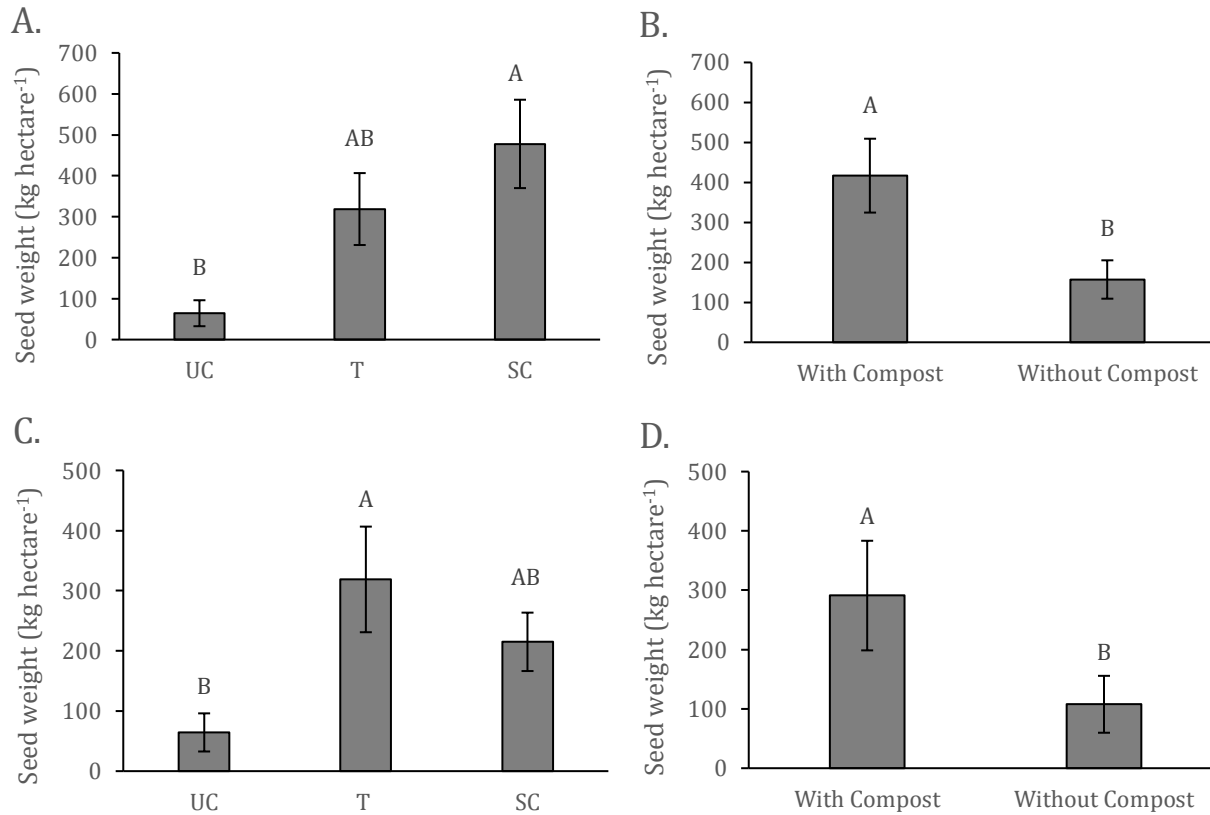


Figure 1. Quinoa seed yield in 2014. Quinoa grown in SC had greater ( $p=0.02$ ) seed weight than UC, with T intermediary (panel A). Quinoa with compost had greater ( $p<0.01$ ) seed weight than those without (panel B). However, when total growing footprint was applied to cropping systems, T had greater seed weight than UC, with SC intermediate ( $p=0.030$ ), and quinoa with compost remained greater than quinoa without compost ( $p=0.007$ ). UC=under-sown clover, T=tillage only, SC=strip crop.

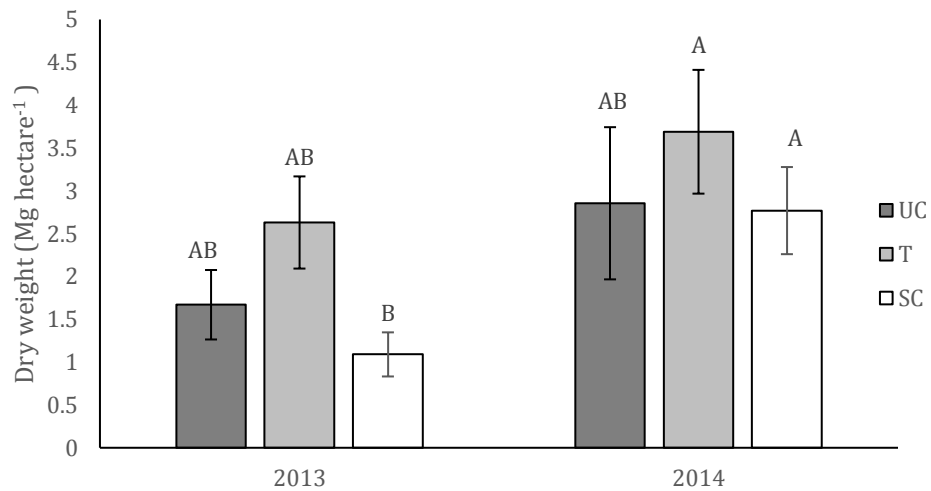


Figure 2. The interaction of cropping system and year was significant for total quinoa biomass ( $p=0.015$ ). T 2014 and SC 2014 were greater than SC 2013 ( $p=0.03$  and  $p<0.01$ , respectively). UC=under-sown clover, T=tillage only, SC=strip crop.

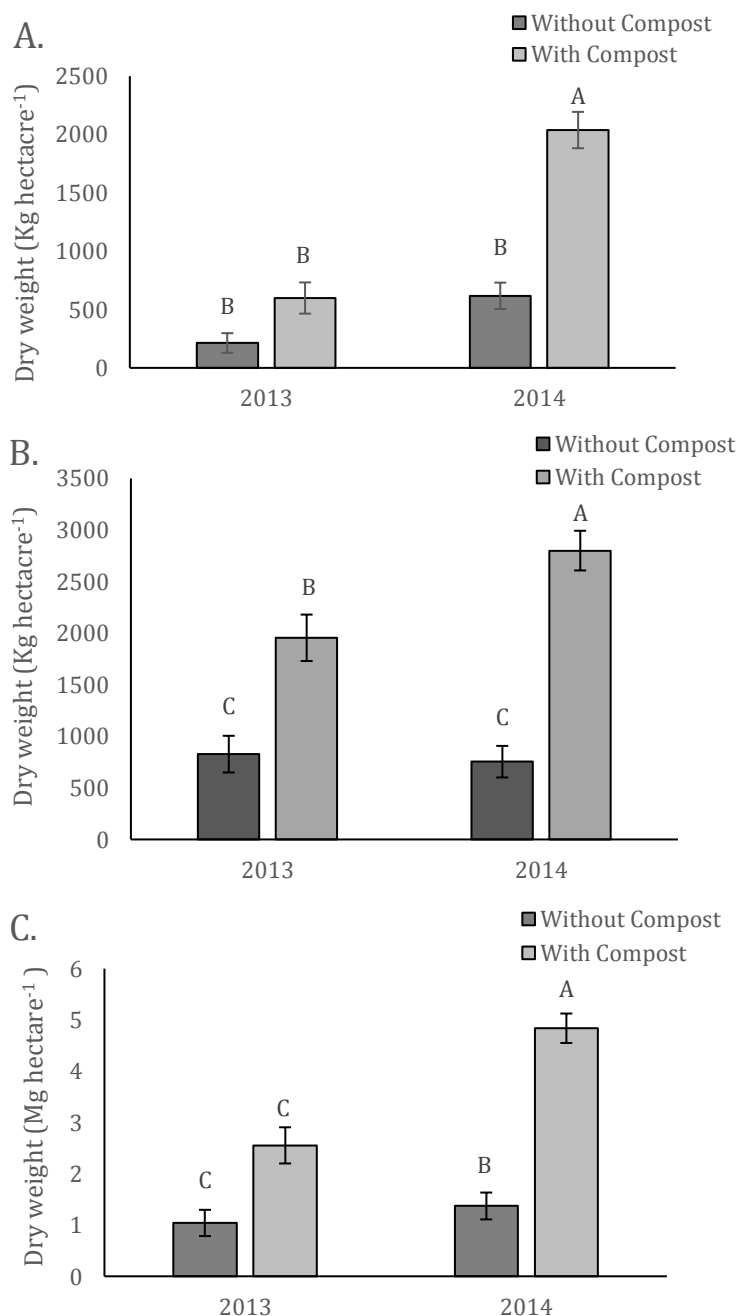


Figure 3. Compost and year interaction was significant for panicles (A) stems (B) and total dry weight (C) ( $p < 0.001$ ,  $p < 0.01$  and  $p = 0.04$ , respectively). Panicles (panel A) were heaviest in +C quinoa in 2014 ( $p < 0.01$ ) over any other compost year combination. 2014+C stems (panel B) had highest dry weights over 2013+C ( $p < 0.01$ ), with both 2013 and 2014 –C lowest ( $p < 0.01$ ) which did not differ from each other. The addition of compost resulted in greater total biomass (panel C) in both years but dry weight in 2014 was greater than 2013 in compost plots ( $p < 0.01$ ).

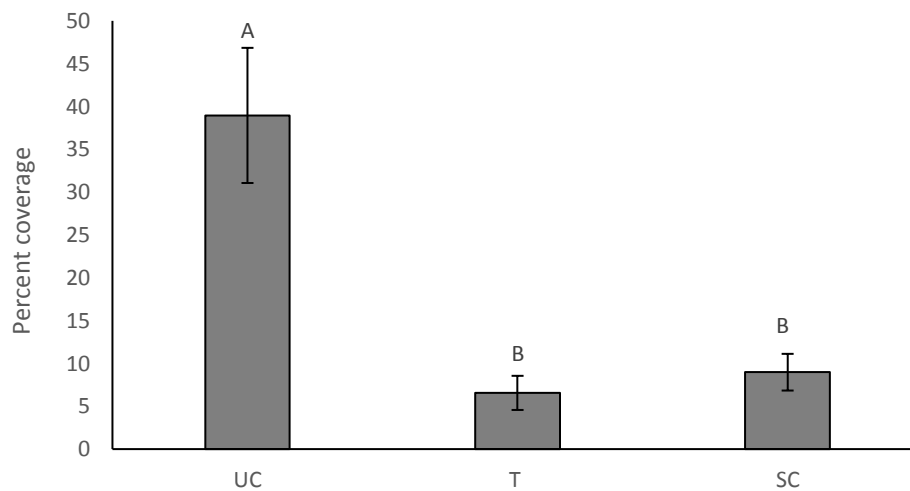


Figure 4. The main effect of treatment was significant ( $p=0.002$ ) in percent weed coverage. UC was greater than both T and SC, which did not differ from each other ( $p=0.002$  and  $p=0.011$ , respectively). UC=under-sown clover, T=tillage only, SC=strip crop.

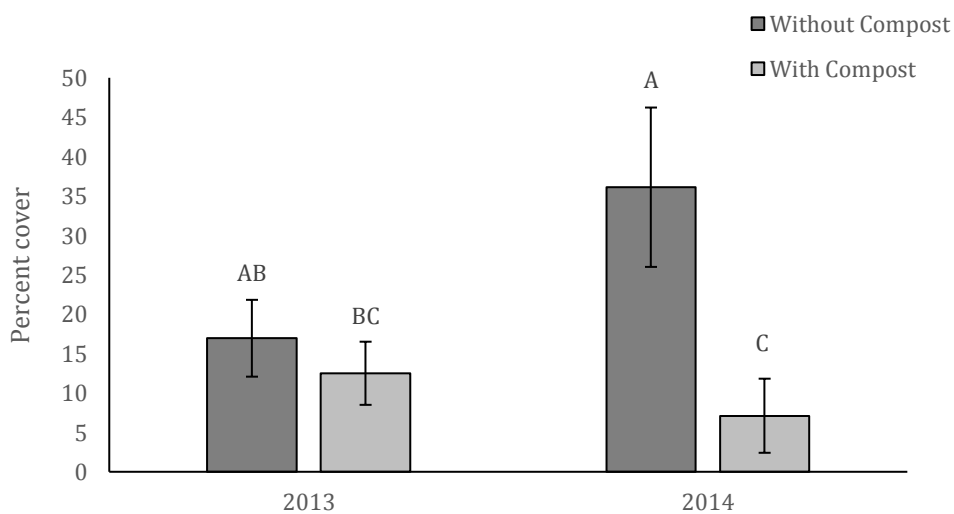


Figure 5. Interaction of compost and year was significant ( $p=0.004$ ) in percent of ground cover measured visually at harvest. 2014-C was greater than both 2014+C and 2013+C ( $p=0.001$  and  $p=0.015$ , respectively). 2013-C was also greater than 2014+C ( $p=0.026$ ).

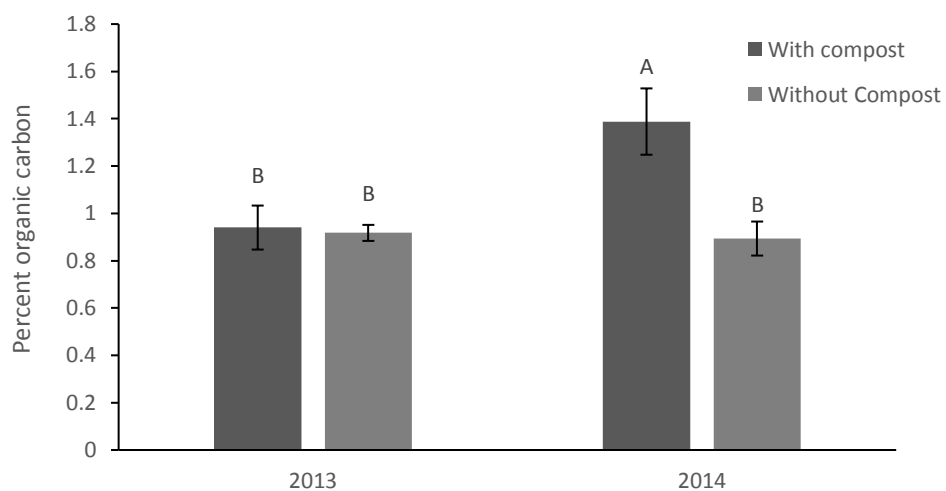


Figure 6. The interaction of compost and year was significant ( $p=0.02$ ). There was more organic carbon in +C soils in 2014 than in any other year-compost combination ( $p<0.01$ ).

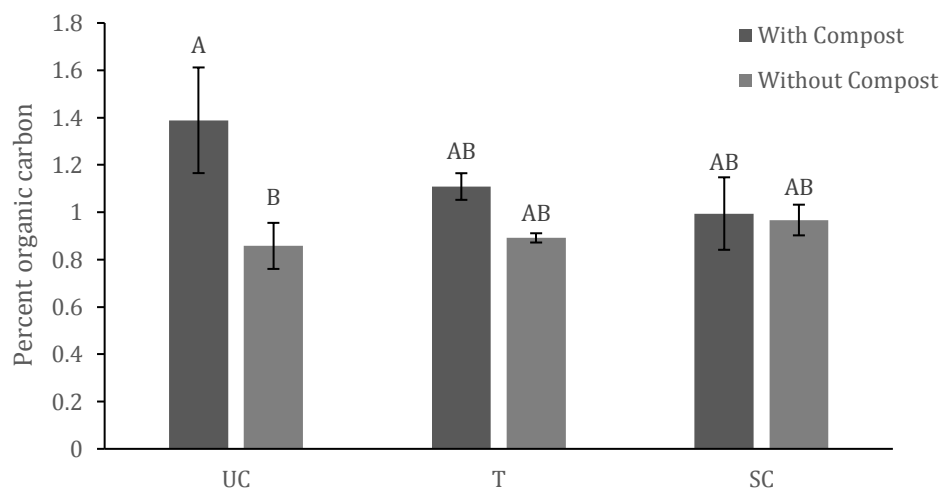


Figure 7. The interaction of cropping system and compost was significant in percentage of total soil organic carbon. UC+C has higher TOC than UC-C ( $p=0.001$ ) with no other differences between cropping system/compost combinations. UC=under-sown clover, T=tillage only, SC=strip crop.

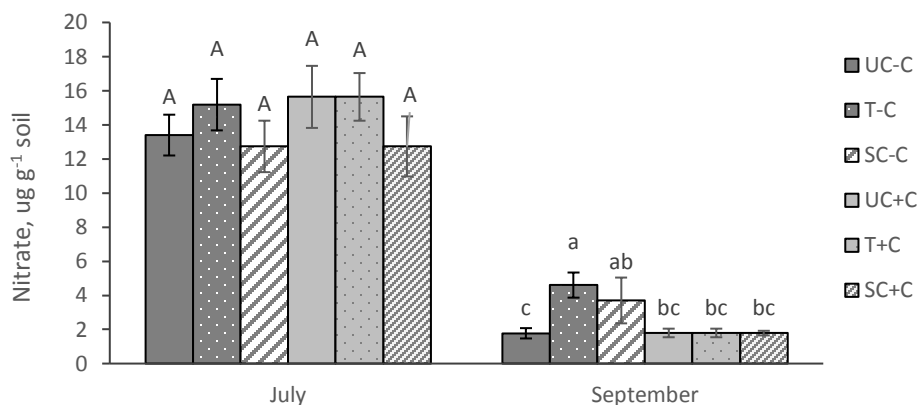


Figure 8. Interaction between cropping system, compost, and month was significant ( $p=0.021$ ) in soil extractable  $\text{NO}_3^-$ . There were no differences between cropping system/compost combinations in July. However, in September, T-C had more  $\text{NO}_3^-$  than T+C, UC+C, SC+C and UC-C ( $p<0.001$  for all comparisons). There was also more  $\text{NO}_3^-$  in SC-C than UC-C in September ( $p=0.05$ ). UC=under-sown clover, T=tillage only, SC=strip crop.

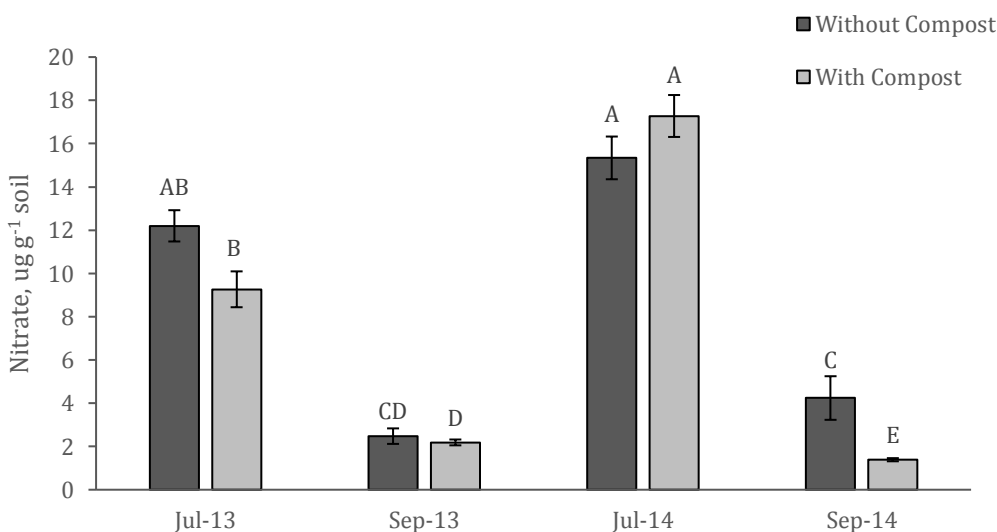


Figure 9. The interaction of compost, year and month was significant ( $p<0.001$ ) for soil nitrate ( $\text{NO}_3^-$ ).  $\text{NO}_3^-$  was higher in July than September ( $p<0.001$ ).  $\text{NO}_3^-$  was lower in July+C 2013 than both +C and -C in July 2014, with -C in July 2013 intermediary ( $p<0.001$  for all comparisons). In September, -C2014 was greater than September +C2013 and +C2014 ( $p=0.038$  and  $p<0.001$ , respectively).  $\text{NO}_3^-$  in September 2014+C was lowest ( $p=0.005$ ).



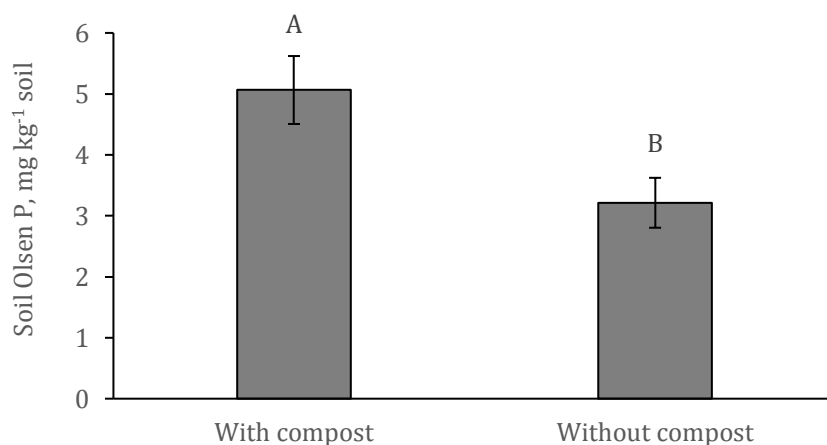


Figure 10. The main effect of compost was significant for soil Olsen P ( $p=0.002$ ), averaged over both years.

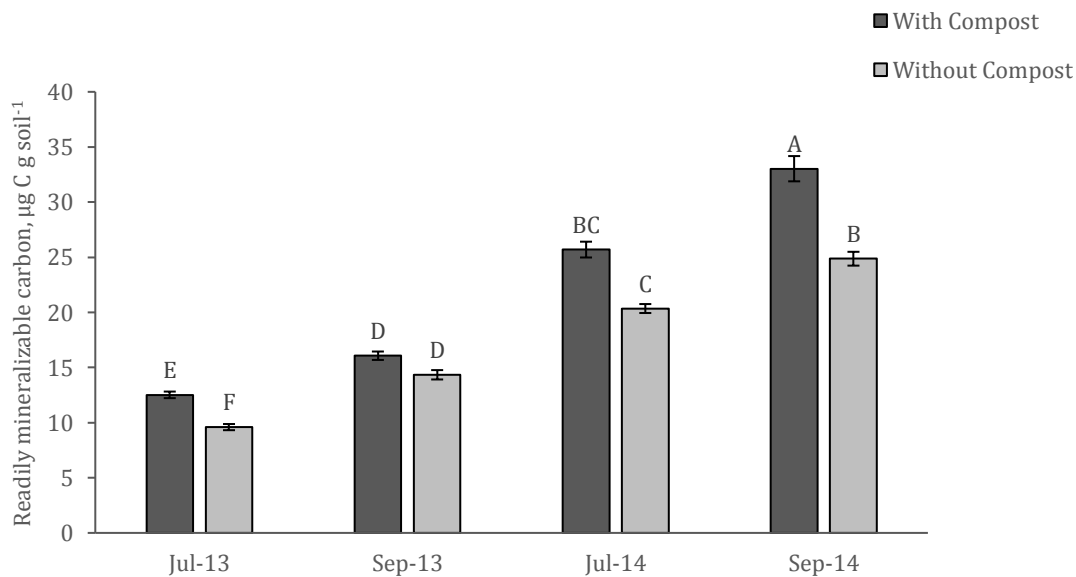


Figure 11. The interaction of compost, year and month was significant ( $p=0.009$ ) for readily mineralizable carbon (RMC). RMC differed ( $p<0.001$ ) in each month, year, and compost combination except in September 2013 where  $-C$  and  $+C$  were the same and in 2014 where  $-C$  in September was the same as  $+C$  in July.

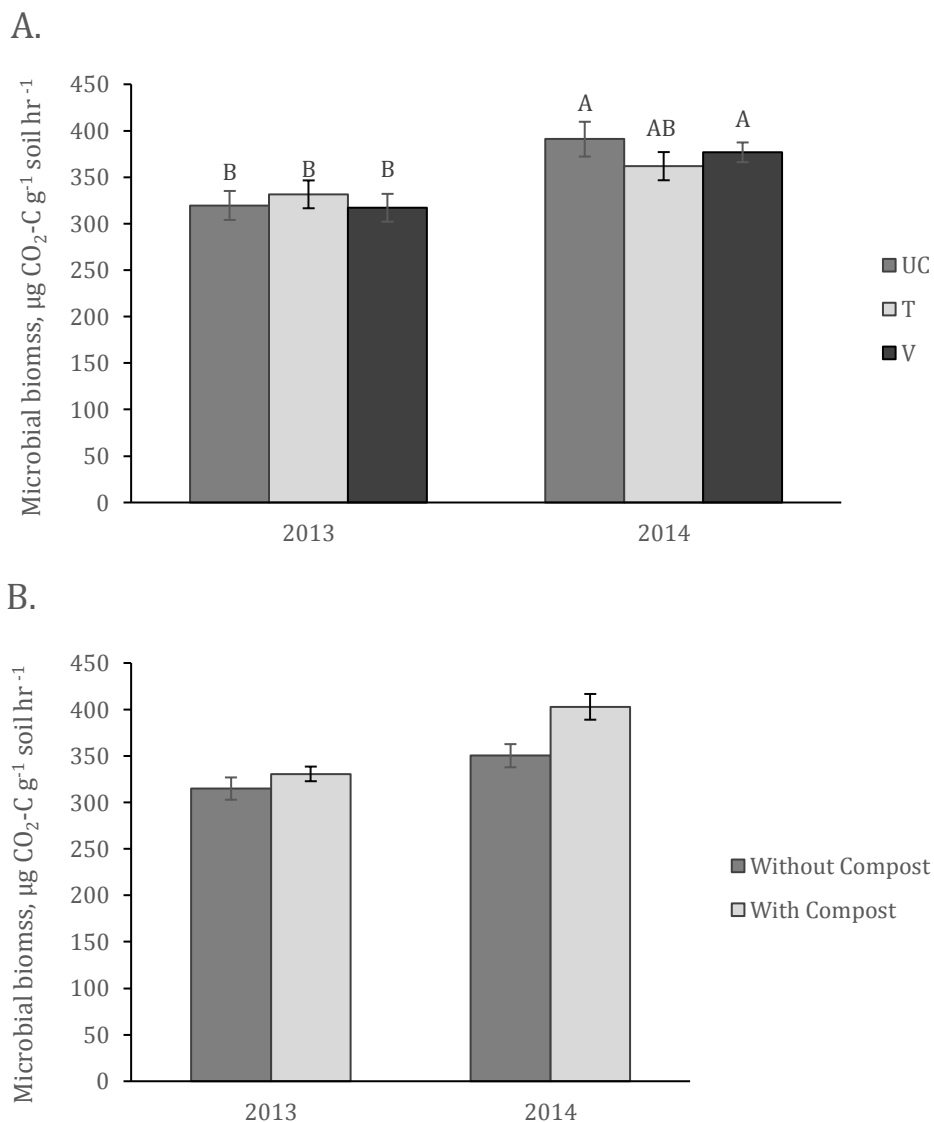


Figure 12. The interactions of cropping system and year (panel A) and compost and year (panel B) were significant ( $p=0.043$  and  $p=0.036$ , respectively) for microbial biomass. There was no cropping system effect within either year but a compost effect was observed in 2014. Microbial biomass was greater in 2014 than 2013 ( $p<0.001$  for all comparisons) except in T treatments which did not differ between years. UC=under-sown clover, T=tillage only, SC=strip crop.

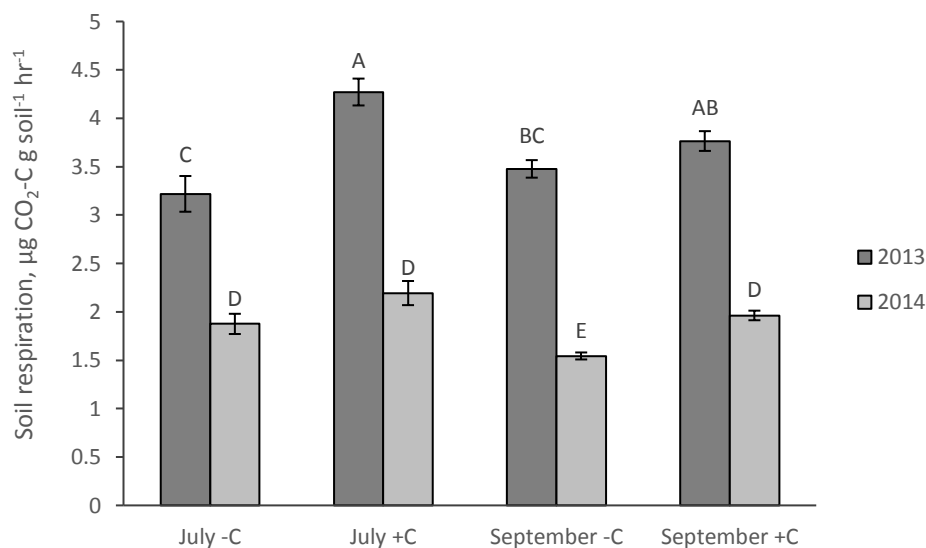


Figure 13. The interaction of compost, year, and month was significant ( $p=0.012$ ) for soil respiration. All comparisons were significant at  $p<0.001$ , except July2013-C differed from Sept 2013+C at  $p=0.0044$  and July 2014-C differed from September 2014-C at  $p=0.054$ .

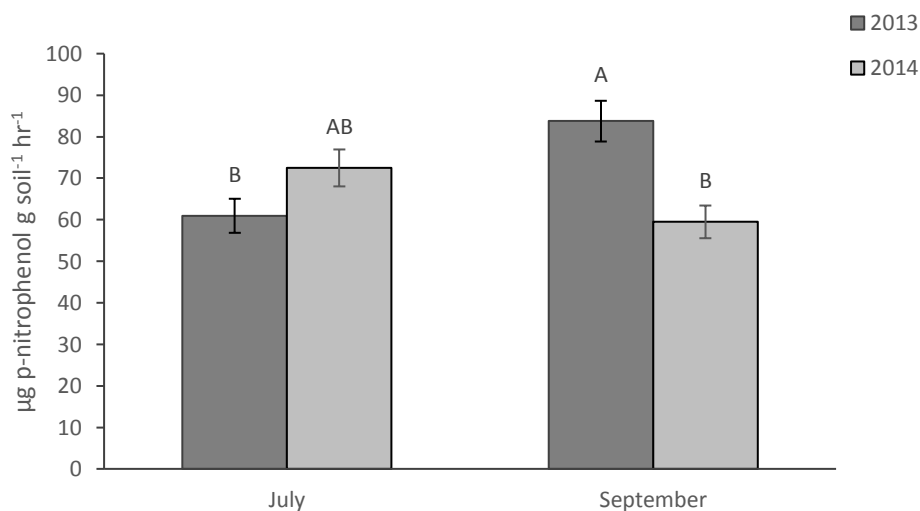


Figure 14. The interaction of year and month are significant ( $p=0.001$ ) for  $\beta$ -glucosaminidase. September 2013 was greater than July 2013 and September 2014, while July 2014 was intermediate ( $p=0.010$  and  $p=0.005$ , respectively).

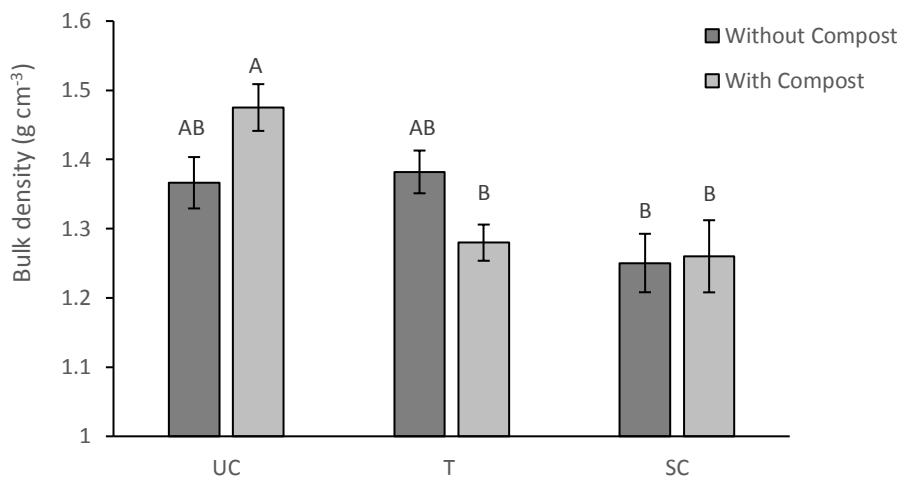


Figure 15. Interaction of cropping system and compost was significant ( $p=0.006$ ) for soil bulk density from 0 to 15 cm. UC+C was greater than T+C, SC-C and SC+C ( $p=0.006$ ,  $p=0.007$ , and  $p=0.014$ , respectively) with UC-C and T-C intermediary. UC=under-sown clover, T=tillage only, SC=strip crop.

## CHAPTER IV: GROWTH RESPONSE OF QUINOA UNDER LINE SOURCE SPRINKLER DESIGN

### Abstract

In the face of increasing water shortages, the western United States could benefit from diversifying crop rotations with crops tolerant to drought conditions. Quinoa (*Chenopodium quinoa* Willd.) has been developed to thrive in a wide range of ecosystems with scarce water resources in South America, yet few varieties are developed for our region. Before widespread adoption of a novel crop is feasible, the difference in varietal response to irrigation rate is critical. This study was conducted as a line source irrigation trial with varieties from commercially available sources and the quinoa breeding program at Washington State University. There was no seed set in either year at any field location due to suspected pollen sterility or seed abort in response to high summer temperatures. Quinoa biomass data suggests optimum water use efficiency at irrigation rates from 23-42 cm in 2013 and limited impact of irrigation rate in 2014 due to high rainfall. Early season rainfall may be sufficient for quinoa growth in the region; however, lack of seed set remains a critical limiting factor to widespread adoption.

### 1. Introduction

Most of the western United States faces increasing water shortages in the coming years. Climate modeling predicts rising temperatures and decreasing precipitation resulting in widespread drought of increasing severity for the western US as the 21st century progresses (Gutzler and Robbins, 2010; Wehner et al., 2011). In this region,

marginal soils with low nutrient availability, low organic matter, low soil moisture, and high salinity are common, which may be exacerbated as drought conditions become widespread in the future. In the face of these challenges, maintaining sustainable farms requires new management strategies. Incorporating an alternative crop that is well adapted to the projected climate and regional field conditions could be a successful approach to increasing the sustainability of farms in the region.

Quinoa, *Chenopodium quinoa* Willd., may be an ideal alternative crop to meet the demands of the Intermountain West. Quinoa is a traditional crop in South America, particularly in the regions around the Andes Mountains where subsistence farming is common (Bhargava et al., 2006; Jacobsen, 2003). Quinoa ecotypes have been developed to thrive in a wide range of environments and have been proven to resist drought and salinity stress (Peterson and Murphy 2015). The demand for quinoa worldwide is great and has resulted in the rapid increase in market value in recent years (DePillis, 2014).

In many traditional cropping systems in South America, quinoa is grown without irrigation. However, in areas with irrigation available, seed production has been reported to increase up to 40% (Geerts et al. 2008). Key indicators of seed quality such as nitrogen and saponin content have also been linked with the amount and quality of irrigation water (Pulvento et al. 2012; Gómez-Caravaca et al. 2012). Plant uptake of applied fertilizers can be greatly affected by irrigation rate and timing, and therefore should be an important focus of ensuring adequate nutrient availability. Quinoa water use ranges from 0.52 to 1.00 times the reference evapotranspiration rate, depending on phenological stage (Magalí Garcia et al. 2003). Drought stress in quinoa can reduce yields and change the timing of maturity (Geerts et al. 2009; Geerts et al. 2008). Response to drought is highly

dependent on variety and the developmental stage in which stress is applied. Drought stress during flowering or grain fill can reduce yields while drought stress during early vegetative stages (both 2-6 and 6-12 leaves stages) have not impacted yields (Geerts et al. 2008). Additionally, drought stress early in the season can lengthen time to flowering but can also hasten maturity if stress occurs after flowering (Geerts et al. 2009, 2008). Optimum irrigation rates for our region have not been described for quinoa and are essential to implementing successful quinoa production.

A line-source sprinkler system was first described by Hanks et al. (1976, 1980) and offers the benefit of irrigation as a continuous variable within a relatively small area. A line source design has been used successfully in many field crops and may prove useful in determining the varietal differences in drought stress for our region (Metin Sezen and Yazar 2006; Hanks et al. 1980). The goal of this study is to identify varietal differences in tolerance to a wide range of drought levels through the use of a line source irrigation design. We tested varieties from a breeding program at Washington State University and commercially available sources. We hypothesized that those varieties with higher tolerance to drought stress will show less growth and yield response to irrigation rate than those without. We also hypothesized that over and under irrigated plants will have poorer plant growth measures than optimally irrigated. The objective was to identify varieties well suited for drought conditions and quantify the irrigation requirements for selected varieties.

## 2. Methods

### 2.1. *Field methods*

The field design was a line source irrigation trial based on that of Hanks (1976) with a single irrigation line through the center of the field. In 2013 and 2014 (field G1 and G2, respectively), plots were located in adjacent fields on the Utah Agricultural Experiment Station (UAES) Greenville farm, near North Logan, UT. In 2014, an additional field (K2) was located at the UAES Kaysville farm in Kaysville, UT, approximately 70 miles south of the Greenville location. Ten varieties in 2013 and eleven varieties in 2014 (Table 1) were planted (May 30, 2013 for G1, April 25, 2014 for G2 and May 6, 2014 for K2) perpendicular to the irrigation line in four replicate blocks in a completely randomized block design. Row spacing of 45 cm and a seeding rate of 13.5 kg ha<sup>-1</sup> were used for all varieties. Due to limited seed availability, two rows of each variety were planted per block in 2013 while three rows of each variety were planted in 2014. Inter-variety spacing was the same 45 cm as intra-variety spacing with two border rows on the top and bottom of the field. Irrigation was applied with over-head sprinklers on 1.8 m tall risers with Nelson Rotator R33 heads (Nelson Irrigation Corporation, Walla Walla, WA). The approximate spray pattern of this head with line pressure at 65 PSI is 15.2 m. Therefore, irrigation rates, soil and plant sampling were determined at positions 3, 6.1, 9.1, 12.2, 15.2, and 18.3 m from the irrigation line to allow for a full range of soil moisture conditions. The sample locations closest to the irrigation line approximated a waterlogged condition while those furthest away received no irrigation and approximated dryland conditions.



The crop was established in early spring with the use of additional overhead sprinkler lines to ensure uniform emergence. A single application of urea at  $112 \text{ kg N ha}^{-1}$  was applied uniformly across the field and watered in with approximately 2.5 cm of water. Line source irrigation was instituted on June 14, 2013 and June 4, 2014 and June 6, 2014 for fields G1, G2, and K2, respectively, and continued until crop maturity in the fall. The duration, frequency, and amount of irrigation were recorded at each sample distance, within each of the four blocks to ensure an even distribution throughout the field (Figure 1). Target irrigation rates were based on the Utah State University weather station maximum evapotranspiration rates for the months of July and August times a factor of 1.2. Thus, locations closest to the irrigation line were over-irrigated at a rate of approximately 2 inches per week (Geerts et al., 2008, 2009).

Soil moisture sampling was accomplished twice per season gravimetrically (July 12 and September 30, 2013 and June 10 and October 1, 2014). Six soil subsamples per sample distance (3, 6.1, 9.1, 12.2, 15.2, and 18.3 m from the irrigation line) in each block were collected from 0 to 30 cm using a 2.5 cm corer and combined in the field. Soils were sieved through a 4 mm screen, stored in re-sealable plastic bags and refrigerated at  $4^\circ\text{C}$  until processing within 10 d. Soils were weighed wet and dried at  $105^\circ\text{C}$  to determine moisture content. Soil EC and pH were measured with a 1:2 soil:water suspension using the same soils as the gravimetric water moisture sampling (Pansu and Gautheyrou, 2006). Soil bulk density was determined using samples obtained with a truck-mounted 4 cm diameter Giddings soil probe. Sections were sampled from 0 to 45 cm in depth from which cores were sectioned into 0 to 10, 10 to 20 and 20 to 30 cm depths. An intact subsection with length of 4 cm within each depth range was transferred to a tin, weighed

wet, and then dried at 105 °C for a minimum of 24 h, or until the decrease in weight due to moisture loss had stopped.

Samples collected as described were also analyzed for nutrients. Nitrate- ( $\text{NO}_3^-$ -N) and ammonium-N ( $\text{NH}_4^+$ -N) were extracted in 1M KCl, and analyzed by Lachat (Quickchem 8500, Hach Company, Loveland, CO) using sulfanilamide and phenate methods, respectively according to manufacturer protocols. Soil P and K levels were measured on randomly selected representative samples collected in July using the Olsen method (Gavlak et al., 2003).

Immediately prior to harvest (September 30, 2013 and October 1, 2014), five plants per plot were measured for overall height from ground level from the center of each plot. A 1-m long section of each of two rows per variety at each 3, 6.1, 9.1, 12.2, 15.2, and 18.3 m from the irrigation line were selected for harvest. In the absence of grain, plants within this section were removed and sectioned into stem and panicle portions. Plant portions were weighed wet, dried and re-weighed, then analyzed for N as described above.

Additional plant growth measures were recorded in 2014 for fields G2 and K2 on two key varieties that demonstrated a divergent response to drought stress in field trials in 2013 (i.e., CO407D and QU629). Both varieties appeared to tolerate drought stress relatively well, maintaining panicle production throughout the range of irrigation levels. However, CO407D had a much shorter main stem with compact panicle, while QU629 was considerably taller with a more open panicle structure. Ten leaf samples were collected from each sample distance for each of these two varieties to determine specific leaf area (SLA), measured as the area of the leaf divided by the fresh weight using a leaf

area meter (LI-3100, LI-COR Biosciences, Lincoln, NE).

In order to capture possible differences in rooting characteristics among varieties and drought stress levels, the same two varieties were selected for root analysis. Soil cores were extracted from 0 to 30 cm immediately adjacent to the quinoa row at each irrigation sampling location in each of two rows following harvest. Soil cores were stored in re-sealable plastic bags at 4 °C until processing. Soils were sieved using a series of sieves with the smallest measuring 355 µm. Root particles were separated, brushed to remove soil and weighed. Root images were acquired with an Epson Expression 10000XL flatbed scanner at 400 dpi resolution then analyzed for root length and diameter using WinRHIZO Pro version 2005b (Regent Instrument Inc., Quebec, Canada G2B 5C3).

## *2.2. Statistical analyses*

Due to the difference in varieties planted in each year and weather interactions, each field was analyzed separately with general linear mixed models. For analysis, fields were divided in half along the irrigation line such that each half contained two blocks. Columns were within these halves parallel to the irrigation line and rows perpendicular to the irrigation line. Distance from the irrigation line was assigned to columns as a repeated measure while varieties were assigned to rows. The response variables of quinoa growth, and soil chemical and physical measures were assessed using analyses of variance with PROC GLIMMIX in the SAS for Windows version 9.4 (SAS Institute, Cary, NC). A mean was computed for all subsamples at each sample location. The covariance structure for repeated measures was compound symmetry based on AICc. Response variables were

square root or log transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. Multiple mean comparisons were adjusted using Tukey's method to control for family-wise Type I error rate. There was little influence of field direction on any variable and this effect is therefore only presented when significant.

### 3. Results

Irrigation rate had a more significant impact on plant growth in 2013 than 2014, likely due to differences in rain events between years. While irrigation rates were similar between field sites, the timing and amount of rain events was drastically different (Figures 1 and 2). In 2013, quinoa received no rainfall between seeding and the establishment of the line source irrigation system. In 2014, the fields were planted much earlier and received nearly 5 cm of rain prior to irrigation, and a total of over 25 cm from rain throughout the season. The rain total was over two times greater in G2 than G1, while similar between G1 and K1. The combined effect of greater rainfall and early planting date reduced the impact of imposed drought stress dramatically. In spite of varied conditions between years, no seed set was observed in any variety, at any site. We suspect frequent daily maximum temperatures above 32 °C during flowering and seed set (Figure 3) resulted in pollen sterility (Peterson and Murphy, 2015) Biomass data is therefore presented as a response to irrigation rate.

#### 3.1. *Quinoa growth in field G1*

The main effects of variety and distance were significant for field G1 on panicle ( $p=0.001$  and  $p=0.004$ , respectively), stem ( $p<0.001$  and  $p=0.001$ , respectively) and total dry weights ( $p<0.001$  and  $p=0.001$ , respectively) (Table 2). Averaged over all

varieties, panicle and stem weights were greater in 3, 6.1, and 9.1 m locations than 15.2 and 18.3 m, with 12.2 intermediate (Table 2). The total biomass of quinoa at 3 m was greater than those plant 12.2 m or farther from the irrigation, and greater biomass at 12.2 m than 18.3 m with no other differences (Table 2). Biomass data suggests the critical optimum irrigation range falls between 9.1 and 12.2 m rates, with no additional benefit for irrigation rates exceeding that observed at 9.1 m.

Averaged over all distances, panicle weights were similar for most varieties in G1 except Cahuil had higher panicle weights than Blanca, CO407, Cherry Vanilla, and Oro de Valle. All other varieties did not differ (Table 2). Stem weights were higher in QQ056 and Faro than Cherry Vanilla, and Blanca, which did not differ from each other. Similarly, QQ056 and Faro had higher total biomass than CO407, Cherry Vanilla, and Blanca. Faro also had greater total biomass than Cherry Vanilla, and Blanca, with all other varieties intermediate. As a measure of resource partitioning, the panicle to total biomass ratio (P:T) was greater in Cahuil than CO407, Oro de Valle, QQ056, QQ74 and Faro (Table 3). Blanca also had higher P:T than Faro, QQ056, and Oro de Valle. No other differences between varieties were observed.

The interaction of distance and month impacted soil available nitrate ( $\text{NO}_3^-$ ) ( $p=0.001$ ) and soil moisture ( $p=0.001$ ). In mid-season, field G1 had greater  $\text{NO}_3^-$  levels at 18.3 m than 6.1 or 3 m while by the end of the season, there was greater  $\text{NO}_3^-$  at 18.3 and 15.2 m than any other distance. This suggests that as the season progressed, quinoa growing under lower irrigation rates was unable to access available soil nitrate. Soil moisture data followed a similar trend. Mid-season, there was more moisture at 3 and 6.1 m than 15.2 and 18.3 m, with 9.1 and 12.2 m from the irrigation source intermediate. By

the end of the season, there was more moisture at 15.2 m than all other distances except 18.3 m, which was similar. There were no differences observed in bulk density measures (data not shown).

Irrigation effects were observed on specific root length (SRL) in field G1 only ( $p=0.028$ ). Averaged over all varieties, quinoa at the furthest distance from the irrigation source (18.3 m) had higher SRL than at 9.1 m, with all other distances intermediate (Table 4). A similar pattern was observed in the total root length and root surface area. Quinoa from 3 to 12.2 m had similar total root lengths while the further distances had less total length (Table 5). Greater surface area was observed in quinoa at distances up to 9.1 m while plants at 15.2 m had the least surface area and other distances intermediate. There was no effect of variety on rooting observed in this field.

### 3.2. *Quinoa growth in field G2*

There was a lack of significant response to irrigation in both 2014 fields. Distance from the irrigation source was a significant factor only for panicle weights (Table 6). Quinoa grown at 3 or 6.1 m from the line had greater panicle mass than quinoa at 15.2 m, with no other differences observed. This suggests a reduced effect of irrigation on development this year.

In field G2, the main effect of variety was significant ( $p=0.046$ ,  $p=0.001$  and  $p=0.002$ , respectively) for panicle, stem, total biomass and P:T ratio (Table 3 and 6). The varieties QQ056 and QU629 had greater stem and total biomass weights than NL-6, KU-2, and Titicaca and likewise greater plant height at harvest along with Oro de Valle and Cherry Vanilla. While the main effect of variety was significant for panicle weights,

when adjusted for multiple comparisons, there were no differences between varieties (Table 3). However, the ratio of panicle to total biomass did differ between varieties with Cahuil and Titicaca greatest. The varieties Cherry Vanilla, CO407, QQ056, QU629, and Oro de Valle had the lowest P:T ratios, with all other intermediate (Table 3).

Soil nitrate in field G2 was not impacted by distance or sample date. Although the amount of total N applied was the same over both years, the lack of treatment effects suggests that moisture was not a limiting factor in nutrient uptake. There were few differences observed in soil moisture as well. The interaction of month and distance was significant ( $p=0.041$ ). Mid-season, soils at 12.2, 15.2 and 18.3 m were drier than those at 3 and 6.1 m but there were no differences between soil moisture late in the season. (Table 7). There were no differences observed in bulk density measures (data not shown).

The interaction of variety and month was significant for specific leaf area (SLA) ( $p<0.001$ ). In field G2, CO407 was greater than QU629 in August, while both varieties in June were greater than QU629 in August (Table 7).

### 3.3. *Quinoa growth in field K1*

Although geographically separated from G2 by approximately 97 km, field K1 also showed a lack of response to irrigation rate in 2014. The stem and total biomass measures in field K1 were impacted by an interaction between distance and variety ( $p=0.002$  and  $p=0.032$ , respectively), indicating either a difference in response to irrigation rate or field nutrient conditions between varieties. There were no differences observed within variety regardless of distance; however, between variety QU629 and CO407, there were differences in both stem and panicle weights (Table 5). Stem weights

were greater in QU629 than CO407 at both 6.1 m and 9.1 m while total biomass was greater in QU629 than CO407 at 9.1 m only. Panicle weights were not different between varieties but were greater at distance 3, 6.1 and 12.2 m than 15.2 m, when averaged over both varieties (Table 5). Analysis of P:T ratio showed a significant interaction between variety and distance ( $p=0.046$ ), yet when adjusted for multiple comparisons using Tukey's method, no significant differences were observed between variety and distance combinations (data not shown). No differences were noted between samples for plant height at harvest. Overall, the biomass collected for this field was much lower than G2. The limited responses to irrigation source distance and much smaller biomass than field G2 suggest growth was limited by other factors such as nutrient availability or weed competition.

The extractable nitrate levels in K1 are much lower than those of G1 or G2 and may explain the low total biomass. Sample month impacted soil available  $\text{NO}_3^-$  levels ( $p=0.018$ ) where mid-season soil samples had greater available  $\text{NO}_3^-$  than late season (Table 6). There were no differences in nitrate levels between distances, suggesting all treatment levels were N deficient. Analysis of soil moisture, revealed a significant interaction between month and distance ( $p<0.001$ ). At mid-season, there was more soil moisture closer to the irrigation source at 3 and 6.1 m compared to the other distances. Soils at 9.1 m had more soil moisture than 15.2 and 18.3 m. By the end of the season, there were no differences in soil moisture.

The interaction of variety and month was significant for specific leaf area (SLA) ( $p=0.003$ ). In field K1, SLA was greater in August than June within each variety, and not different between varieties within each sample date (Table 9).



#### 4. Discussion

The differences in field conditions between seasons resulted in a lack of response to irrigation in 2014 at field G2. When subjected to drought conditions in 2013, quinoa biomass was optimal and between 9.1 to 12.2 m distance, which equates to between 23 and 41 cm of water applied for the season. In 2014, the treatments with the least irrigation applied were near this optimal range due to higher rainfall totals, which explains the lack of response to line source treatments in general. It has been shown that dryland quinoa can be successful with rainfall totals far below this rate (González et al., 2015; Martínez et al., 2009).

Our hypothesis that varietal differences would dictate growth response to irrigation rate was not supported as we observed few differences between varieties. The general lack of variety by distance interactions indicates a similar response between varieties tested to field conditions. Bertero and Ruiz (2008) report differences in biomass between several varieties also presented in this study (i.e., NL-6, CO407, and Faro); however, the total biomass in our study was 2 to 4 times lower at any irrigation rate. The discrepancy in biomass from previous studies suggests irrigation rate alone was not the only factor responsible for quinoa growth. While total biomass indicates some degree of differences among varieties for drought tolerance, biomass partitioning may be more useful. In G1, Cahuil had greater panicle weights, which may be an indicator of high seed yield potential. Quinoa biomass during flowering and grain filling stages is well correlated with seed number (Bertero and Ruiz, 2008), but total yield may be more accurately predicted by biomass at other stages of growth (Bertero and Ruiz, 2010). Gonzales et al. (2009) showed similar rates of biomass partitioning coefficients with

variable irrigation levels. In our study, the panicle to total biomass ratios showed a similar response with no differences observed between irrigation levels, only between varieties. However, in the absence of seed production, we cannot confirm that these results would be an adequate predictor of total yield.

Not only was rain a major factor in the response of quinoa to irrigation, but peak summer temperatures also hampered our results. Although Peterson and Murphy (2015) reported increases in yield in response to irrigation during heat stress, we did not observe any benefits of increased irrigation on seed set in any field. We observed field conditions other than irrigation likely caused the differences in quinoa response. Martinez et al. (2009) also reported yield did not directly correlate to irrigation rates over diverse quinoa varieties; instead, a lower irrigation rate coupled with organic matter inputs yielded highest. The high water inputs in 2014 decreased soil nitrate levels in field G2 while the low soil nitrate levels in field K1 may have resulted from an interaction between previous cropping history and intense weed competition.

Specific root length has been used to describe plant response to environmental conditions such as drought and temperature as well as nutrient availability or presence of soil borne toxins (Ostonen et al., 2007). The quinoa varieties showed less root development in field G1 (i.e., root length, surface area, and specific root length) in drier soils located at greater distances from the irrigation source. Martinez et al. (2009) also reported changes in rooting characteristics of quinoa in response to irrigation treatments. In that study, quinoa that received no irrigation had soil moisture located only at shallow depths and resulted in roots that were longer horizontally in contrast to a main vertical taproot which is more commonly observed (Martínez et al., 2009). We cannot determine,

however, whether our results are due to reduced growth from drought or from changes in morphology because our sampling method only sampled a vertical core from 0 to 30 cm. Gonzalez et al. (2009) reported a decrease in plant and root dry weights in response to both waterlogging and drought stress, which demonstrates the importance of optimizing irrigation for maximum yield potential.

## 5. Conclusion

Water is a valuable resource and increasingly in short supply in the Intermountain West. Drought tolerant crops may be important in keeping farms sustainable throughout the region. Introducing drought tolerant varieties of quinoa as a novel crop in rotation could be a valuable tool for local growers. Quinoa was impacted by the irrigation treatments in one of two years, but was unresponsive due to excessive rainfall in the second season. When drought stressed, quinoa's ideal irrigation rate ranged from 23-42 cm water. Suspected heat intolerance during flowering and seed set resulted in no seed yield in any year. The future of quinoa in this region is dependent on the demonstration of reliable seed production.

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## Tables and figures

**Table 1**

Variety selections by field and year. G1=field UAES Greenville 2013, G2=field UAES Greenville 2014, K1=field UAES Kaysville 2014

| Variety      | Field                   |  |
|--------------|-------------------------|--|
|              | G1 <sup>1</sup><br>2013 | G2 <sup>2</sup> and<br>K1 <sup>3</sup><br>2014 |
| Black        | X                       | X  |
| Blanca       | X                       | X  |
| Cahuil       | X                       | X  |
| Cherry       | X                       | X  |
| Vanilla      |                         |  |
| CO-407       | X                       | X  |
| Faro         | X                       |  |
| KU2          |                         | X  |
| NL-6         |                         | X  |
| Oro de Valle | X                       | X  |
| QQ056        | X                       |  |
| QQ74         | X                       | X  |
| QU629        | X                       | X  |
| Titicaca     |                         | X  |

<sup>1</sup>G1 is located at UAES Greenville in 2013

<sup>2</sup>G2 is located at UAES Greenville in 2014

<sup>3</sup>K1 is located at UAES Kaysville in 2014

**Table 2**

Means (n=4) and p-values for panicle, stem and total biomass dry weights for UAES Greenville farm 2013. Significant differences ( $p < 0.05$ ) are designated by different letters.

|                     | Panicle<br>(g)     | Stem<br>(g)           | Total<br>(g)         |
|---------------------|--------------------|-----------------------|----------------------|
| <b>Variety</b>      |                    |                       |                      |
| Black               | 71.2 <sup>AB</sup> | 166 <sup>AB</sup>     | 236.83 <sup>AB</sup> |
| Blanca              | 56.2 <sup>B</sup>  | 108 <sup>C</sup>      | 168.96 <sup>B</sup>  |
| Cahuil              | 95.4 <sup>A</sup>  | 164.90 <sup>ABC</sup> | 260.33 <sup>AB</sup> |
| CO407               | 53.6 <sup>B</sup>  | 147.10 <sup>ABC</sup> | 200.66 <sup>AB</sup> |
| Cherry Vanilla      | 54.0 <sup>B</sup>  | 131.02 <sup>BC</sup>  | 180.72 <sup>B</sup>  |
| Faro                | 62.9 <sup>AB</sup> | 214.15 <sup>A</sup>   | 277.05 <sup>A</sup>  |
| Oro de Valle        | 55.3 <sup>B</sup>  | 160.73 <sup>ABC</sup> | 216.02 <sup>AB</sup> |
| QQ056               | 65.1 <sup>AB</sup> | 231.59 <sup>A</sup>   | 296.66 <sup>A</sup>  |
| QQ74                | 64.7 <sup>AB</sup> | 168.85 <sup>ABC</sup> | 233.51 <sup>AB</sup> |
| QU629               | 76.8 <sup>AB</sup> | 175.74 <sup>ABC</sup> | 252.53 <sup>AB</sup> |
| <b>Distance (m)</b> |                    |                       |                      |
| 3.0                 | 88.2 <sup>A</sup>  | 271.36 <sup>A</sup>   | 359.57 <sup>A</sup>  |
| 6.1                 | 90.0 <sup>A</sup>  | 226.22 <sup>A</sup>   | 316.26 <sup>AB</sup> |
| 9.1                 | 87.2 <sup>A</sup>  | 230.55 <sup>A</sup>   | 317.77 <sup>AB</sup> |
| 12.2                | 58.9 <sup>AB</sup> | 122.43 <sup>AB</sup>  | 181.32 <sup>BC</sup> |
| 15.2                | 41.3 <sup>BC</sup> | 82.27 <sup>B</sup>    | 123.59 <sup>CD</sup> |
| 18.3                | 27.5 <sup>C</sup>  | 67.86 <sup>B</sup>    | 97.08 <sup>D</sup>   |
| <b>p-values</b>     |                    |                       |                      |
| Variety             | 0.0014             | 0.0023                | 0.0009               |
| Distance            | 0.0201             | 0.0032                | 0.0048               |
| Variety*distance    | 0.5916             | 0.5010                | 0.5232               |

**Table 3**

The mean value of the ratio of panicle to total biomass ratio (P:T) for each field. Significant differences ( $p < 0.05$ ) are designated by different letters. Direction of the field in relation to the irrigation line was significant in field G1 only.

| <b>Field</b>     | <b>G1<sup>1</sup></b> | <b>G2<sup>2</sup></b> | <b>G2<sup>2</sup></b> |
|------------------|-----------------------|-----------------------|-----------------------|
|                  | <b>P:T</b>            | <b>P:T</b>            | <b>Height (cm)</b>    |
| <b>Variety</b>   |                       |                       |                       |
| Black            | 0.301 <sup>ABC</sup>  | 0.312 <sup>ABC</sup>  | 106 <sup>ABC</sup>    |
| Blanca           | 0.323 <sup>AB</sup>   | 0.355 <sup>AB</sup>   | 94.3 <sup>ABC</sup>   |
| Cahuil           | 0.366 <sup>A</sup>    | 0.368 <sup>A</sup>    | 102 <sup>ABC</sup>    |
| Cherry           | 0.311 <sup>ABC</sup>  | 0.282 <sup>BC</sup>   | 130 <sup>A</sup>      |
| Vanilla          |                       |                       |                       |
| CO407            | 0.287 <sup>BCDE</sup> | 0.258 <sup>C</sup>    | 123 <sup>AB</sup>     |
| Faro             | 0.244 <sup>DE</sup>   | -                     | -                     |
| KU2              | -                     | 0.359 <sup>AB</sup>   | 79.0 <sup>C</sup>     |
| QQ056            | 0.230 <sup>E</sup>    | 0.232 <sup>C</sup>    | 133 <sup>A</sup>      |
| QQ74             | 0.299 <sup>BCD</sup>  | -                     | -                     |
| QU629            | 0.308 <sup>ABC</sup>  | 0.233 <sup>C</sup>    | 140 <sup>A</sup>      |
| Oro de Valle     | 0.271 <sup>CDE</sup>  | 0.265 <sup>C</sup>    | 131 <sup>A</sup>      |
| NL6              | -                     | 0.361 <sup>AB</sup>   | 83.9 <sup>BC</sup>    |
| Titicaca         | -                     | 0.374 <sup>A</sup>    | 84.5 <sup>C</sup>     |
| <b>Direction</b> |                       |                       |                       |
| Left             | 0.244 <sup>B</sup>    |                       |                       |
| Right            | 0.329 <sup>A</sup>    |                       |                       |
| <b>p-values</b>  |                       |                       |                       |
| Variety          | <0.001                | <0.001                | <0.001                |
| Direction        | 0.038                 | 0.422                 | 0.608                 |

<sup>1</sup>G1 is located at UAES Greenville in 2013

<sup>2</sup>G2 is located at UAES Greenville in 2014



**Table 4**

Specific root length for field G1 UAES Greenville 2013. Significant differences ( $p < 0.05$ ) are designated by different letters.

| <b>Field G1</b>     | <b>Specific<br/>root length</b> |
|---------------------|---------------------------------|
| <b>Distance (m)</b> |                                 |
| 3.0                 | 1640 <sup>AB</sup>              |
| 6.1                 | 2180 <sup>AB</sup>              |
| 9.1                 | 1390 <sup>B</sup>               |
| 12.2                | 2360 <sup>AB</sup>              |
| 15.2                | 1620 <sup>AB</sup>              |
| 18.3                | 2860 <sup>A</sup>               |
| <b>p-values</b>     |                                 |
| Variety             | 0.848                           |
| Distance            | 0.028                           |
| Variety*distance    | 0.252                           |

**Table 5**

The mean value for root measurements for fields G1 and G2. Significant differences ( $p < 0.05$ ) are designated by different letters.

| <b>Field</b>        | <b>G1<sup>1</sup></b> | <b>G1<sup>1</sup></b> | <b>G2<sup>2</sup></b> | <b>G2<sup>2</sup></b> |
|---------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                     | Total length          | Surface Area          | Total length          | Surface Area          |
| <b>Variety</b>      |                       |                       |                       |                       |
| CO407               |                       |                       | 420 <sup>B</sup>      | 37.3 <sup>B</sup>     |
| QU629               |                       |                       | 687 <sup>A</sup>      | 63.1 <sup>A</sup>     |
| <b>Distance (m)</b> |                       |                       |                       |                       |
| 3.0                 | 1000 <sup>A</sup>     | 146 <sup>A</sup>      |                       |                       |
| 6.1                 | 1050 <sup>A</sup>     | 140 <sup>A</sup>      |                       |                       |
| 9.1                 | 1180 <sup>A</sup>     | 168 <sup>A</sup>      |                       |                       |
| 12.2                | 890 <sup>A</sup>      | 114 <sup>AB</sup>     |                       |                       |
| 15.2                | 498 <sup>B</sup>      | 55.5 <sup>C</sup>     |                       |                       |
| 18.3                | 526 <sup>B</sup>      | 62.0 <sup>BC</sup>    |                       |                       |
| <b>p-values</b>     |                       |                       |                       |                       |
| Variety             | 0.261                 | 0.347                 | <0.001                | <0.001                |
| Distance            | 0.001                 | 0.004                 | 0.169                 | 0.405                 |

<sup>1</sup>G1 is located at UAES Greenville in 2013

<sup>2</sup>G2 is located at UAES Greenville in 2014

<sup>3</sup>K1 is located at UAES Kaysville in 2014

**Table 6**

The main effect of variety and distance on biomass for field G2, UAES Greenville 2014. Significant differences ( $p < 0.05$ ) are designated by different letters. Although the main effect of variety was significant for panicle dry weight, when Tukey's method for multiple comparisons was applied to means comparisons, there were no significant differences for panicle dry weight as shown below.

|                     | Panicle<br>(g)     | Stem<br>(g)       | Total<br>(g)      |
|---------------------|--------------------|-------------------|-------------------|
| <b>Variety</b>      |                    |                   |                   |
| Black               | 80.8 <sup>A</sup>  | 181 <sup>AB</sup> | 262 <sup>AB</sup> |
| Blanca              | 78.1 <sup>A</sup>  | 140 <sup>AB</sup> | 218 <sup>AB</sup> |
| Cahuil              | 98.5 <sup>A</sup>  | 169 <sup>AB</sup> | 268 <sup>AB</sup> |
| CO407               | 51.1 <sup>A</sup>  | 154 <sup>AB</sup> | 205 <sup>AB</sup> |
| Cherry Vanilla      | 64.3 <sup>A</sup>  | 170 <sup>AB</sup> | 235 <sup>AB</sup> |
| KU-2                | 52.1 <sup>A</sup>  | 99.2 <sup>B</sup> | 151 <sup>B</sup>  |
| NL-6                | 59.1 <sup>A</sup>  | 103 <sup>B</sup>  | 162 <sup>B</sup>  |
| Oro de Valle        | 58.3 <sup>A</sup>  | 164 <sup>AB</sup> | 222 <sup>AB</sup> |
| QQ056               | 82.9 <sup>A</sup>  | 257 <sup>A</sup>  | 340 <sup>A</sup>  |
| QU629               | 84.5 <sup>A</sup>  | 270 <sup>A</sup>  | 355 <sup>A</sup>  |
| Titicaca            | 58.8 <sup>A</sup>  | 113 <sup>B</sup>  | 172 <sup>B</sup>  |
| <b>Distance (m)</b> |                    |                   |                   |
| 3.0                 | 99.9 <sup>A</sup>  |                   |                   |
| 6.1                 | 91.5 <sup>A</sup>  |                   |                   |
| 9.1                 | 75.2 <sup>AB</sup> |                   |                   |
| 12.2                | 60.8 <sup>AB</sup> |                   |                   |
| 15.2                | 42.1 <sup>B</sup>  |                   |                   |
| 18.3                | 49.5 <sup>AB</sup> |                   |                   |
| <b>p-values</b>     |                    |                   |                   |
| Variety             | 0.046              | 0.001             | 0.002             |
| Distance            | 0.022              | 0.252             | 0.128             |
| Variety*distance    | 0.113              | 0.194             | 0.073             |

**Table 7**

Means (n=4) and p-values for panicle, stem and total biomass dry weights for field K1 UAES Kaysville 2014. Significant differences ( $p < 0.05$ ) are designated by different letters.

|                         | Panicle dry weight<br>(g) | Stem dry weight<br>(g) | Total dry weight<br>(g) |                    |                     |
|-------------------------|---------------------------|------------------------|-------------------------|--------------------|---------------------|
| <b>Variety</b>          |                           |                        |                         |                    |                     |
| CO407                   | 22.0                      | 44.1                   | 66.0                    |                    |                     |
| QU629                   | 30.8                      | 67.6                   | 98.3                    |                    |                     |
| <b>Distance (m)</b>     |                           |                        |                         |                    |                     |
| 3.0                     | 32.6 <sup>A</sup>         |                        |                         |                    |                     |
| 6.1                     | 32.6 <sup>A</sup>         |                        |                         |                    |                     |
| 9.1                     | 29.1 <sup>AB</sup>        |                        |                         |                    |                     |
| 12.2                    | 27.3 <sup>AB</sup>        |                        |                         |                    |                     |
| 15.2                    | 17.0 <sup>B</sup>         |                        |                         |                    |                     |
| 18.3                    | 19.5 <sup>AB</sup>        |                        |                         |                    |                     |
| <b>Variety*distance</b> |                           | <b>CO407</b>           | <b>QU629</b>            | <b>CO407</b>       | <b>QU629</b>        |
| 3.0                     |                           | 47.0 <sup>BE</sup>     | 82.3 <sup>ACD</sup>     | 77.3 <sup>AB</sup> | 117.3 <sup>AB</sup> |
| 6.1                     |                           | 44.3 <sup>DE</sup>     | 88.2 <sup>ABC</sup>     | 71.6 <sup>AB</sup> | 126.0 <sup>AB</sup> |
| 9.1                     |                           | 36.9 <sup>CE</sup>     | 82.8 <sup>ABD</sup>     | 57.2 <sup>B</sup>  | 120.7 <sup>A</sup>  |
| 12.2                    |                           | 45.4 <sup>ABCD</sup>   | 59.4 <sup>ABCD</sup>    | 68.1 <sup>AB</sup> | 91.3 <sup>AB</sup>  |
| 15.2                    |                           | 36.8 <sup>ABCD</sup>   | 41.9 <sup>ABCD</sup>    | 51.5 <sup>AB</sup> | 61.2 <sup>AB</sup>  |
| 18.3                    |                           | 54.0 <sup>ABCD</sup>   | 50.8 <sup>ABCD</sup>    | 70.5 <sup>AB</sup> | 73.3 <sup>AB</sup>  |
| <b>p-values</b>         |                           |                        |                         |                    |                     |
| Variety                 | 0.159                     | 0.076                  |                         |                    | 0.145               |
| Distance                | 0.023                     | 0.680                  |                         |                    | 0.387               |
| Variety*distance        | 0.708                     | 0.002                  |                         |                    | 0.032               |

**Table 8**

Soil nitrate (NO<sub>3</sub><sup>-</sup>) and soil moisture content for all fields. Treatment means designated with different letters are significant at p<0.05.

|                               | NO <sub>3</sub> <sup>-</sup> |                 |                   | Moisture           |                      |                      |
|-------------------------------|------------------------------|-----------------|-------------------|--------------------|----------------------|----------------------|
|                               | G1 <sup>1</sup>              | G2 <sup>2</sup> | K1 <sup>3</sup>   | G1 <sup>1</sup>    | G2 <sup>2</sup>      | K1 <sup>3</sup>      |
| <b>Month</b>                  |                              |                 |                   |                    |                      |                      |
| Mid-Season                    |                              | 8.86            | 3.88 <sup>A</sup> |                    |                      |                      |
| Late-season                   |                              | 4.09            | 1.11 <sup>B</sup> |                    |                      |                      |
| <b>Distance*month<br/>(m)</b> |                              |                 |                   |                    |                      |                      |
| Mid-Season                    |                              |                 |                   |                    |                      |                      |
| 3.0                           | 8.61 <sup>BC</sup>           |                 |                   | 12.4 <sup>B</sup>  | 13.6 <sup>A</sup>    | 12.6 <sup>A</sup>    |
| 6.1                           | 10.9 <sup>ABC</sup>          |                 |                   | 11.8 <sup>B</sup>  | 12.6 <sup>AB</sup>   | 12.3 <sup>A</sup>    |
| 9.1                           | 17.3 <sup>AB</sup>           |                 |                   | 10.4 <sup>B</sup>  | 11.6 <sup>ABCD</sup> | 9.66 <sup>BCD</sup>  |
| 12.2                          | 20.2 <sup>AB</sup>           |                 |                   | 8.34 <sup>BC</sup> | 10.5 <sup>CDE</sup>  | 7.88 <sup>DE</sup>   |
| 15.2                          | 28.1 <sup>AB</sup>           |                 |                   | 6.68 <sup>C</sup>  | 9.51 <sup>E</sup>    | 7.24 <sup>E</sup>    |
| 18.3                          | 36.0 <sup>AB</sup>           |                 |                   | 6.78 <sup>C</sup>  | 10.0 <sup>DE</sup>   | 8.12 <sup>DE</sup>   |
| Late-season                   |                              |                 |                   |                    |                      |                      |
| 3.0                           | 2.44 <sup>C</sup>            |                 |                   | 27.5 <sup>A</sup>  | 12.7 <sup>ABC</sup>  | 11.0 <sup>ABC</sup>  |
| 6.1                           | 2.03 <sup>C</sup>            |                 |                   | 26.7 <sup>A</sup>  | 12.6 <sup>ABC</sup>  | 11.1 <sup>AB</sup>   |
| 9.1                           | 2.28 <sup>C</sup>            |                 |                   | 28.8 <sup>A</sup>  | 12.3 <sup>ABC</sup>  | 11.0 <sup>ABC</sup>  |
| 12.2                          | 9.83 <sup>ABC</sup>          |                 |                   | 27.1 <sup>A</sup>  | 11.7 <sup>ABCD</sup> | 9.94 <sup>ABCD</sup> |
| 15.2                          | 30.0 <sup>AB</sup>           |                 |                   | 34.5 <sup>A</sup>  | 11.1 <sup>BCDE</sup> | 8.96 <sup>BCDE</sup> |
| 18.3                          | 46.7 <sup>A</sup>            |                 |                   | 31.5 <sup>A</sup>  | 11.1 <sup>BCDE</sup> | 8.66 <sup>CDE</sup>  |
| <b>p-values</b>               |                              |                 |                   |                    |                      |                      |
| Distance                      | 0.001                        | 0.398           | 0.139             | 0.029              | 0.001                | <0.001               |
| month                         | 0.029                        | 0.055           | 0.018             | 0.004              | 0.089                | 0.116                |
| Distance*month                | 0.026                        | 0.337           | 0.122             | 0.002              | 0.041                | 0.009                |

<sup>1</sup>G1 is located at UAES Greenville in 2013

<sup>2</sup>G2 is located at UAES Greenville in 2014

<sup>3</sup>K1 is located at UAES Kaysville in 2014

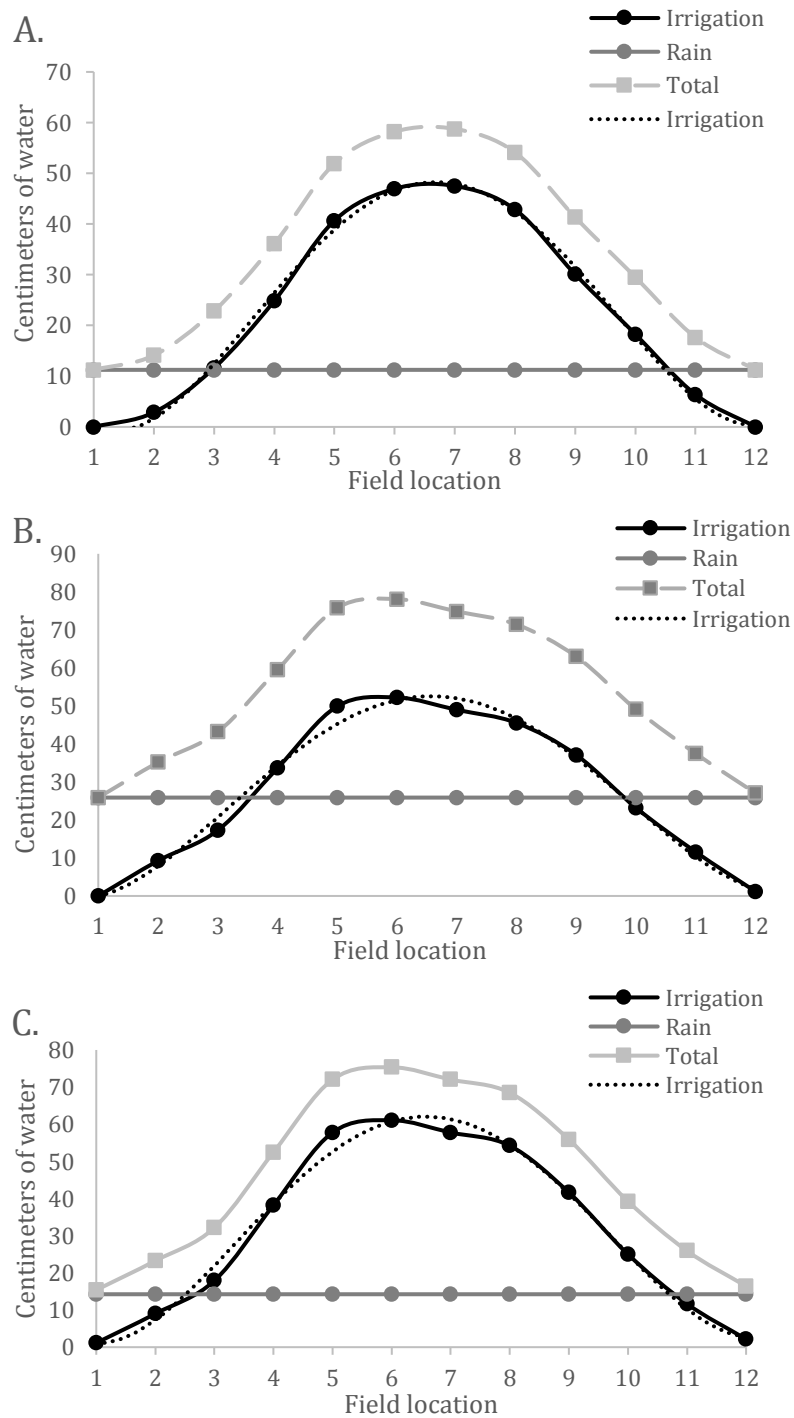
**Table 9**

Specific leaf area for two varieties in 2014. Means designated by different letters represent significant differences at  $p < 0.05$ .

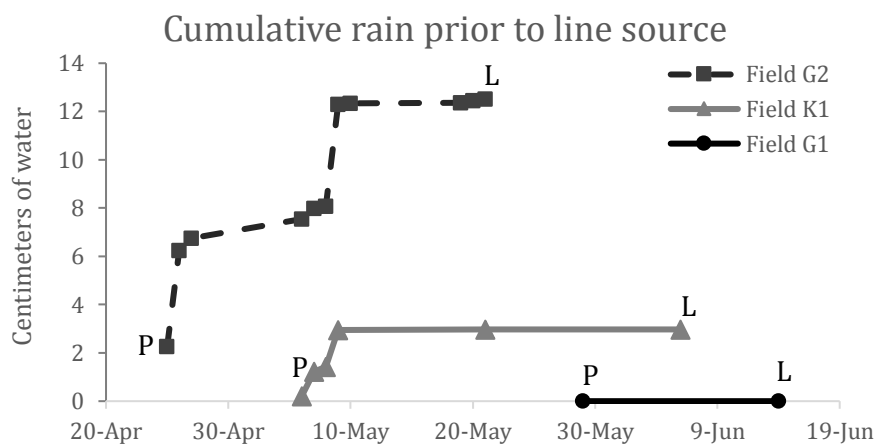
| Variety*month          | Specific leaf area  |                     |
|------------------------|---------------------|---------------------|
|                        | G2 <sup>1</sup>     | K2 <sup>2</sup>     |
| June                   |                     |                     |
| CO407                  | 0.042 <sup>A</sup>  | 0.042 <sup>BC</sup> |
| QU629                  | 0.042 <sup>AB</sup> | 0.042 <sup>C</sup>  |
| August                 |                     |                     |
| CO407                  | 0.039 <sup>B</sup>  | 0.051 <sup>A</sup>  |
| QU629                  | 0.034 <sup>C</sup>  | 0.046 <sup>AB</sup> |
| <b>p-values</b>        |                     |                     |
| Variety                | <0.001              | 0.222               |
| Distance               | 0.605               | 0.085               |
| month                  | <0.001              | <0.001              |
| Variety*distance       | 0.563               | 0.409               |
| Variety*month          | <0.001              | 0.003               |
| Distance*month         | 0.780               | 0.461               |
| Variety*distance*month | 0.838               | 0.812               |

<sup>1</sup>G2 is located at UAES Greenville in 2014

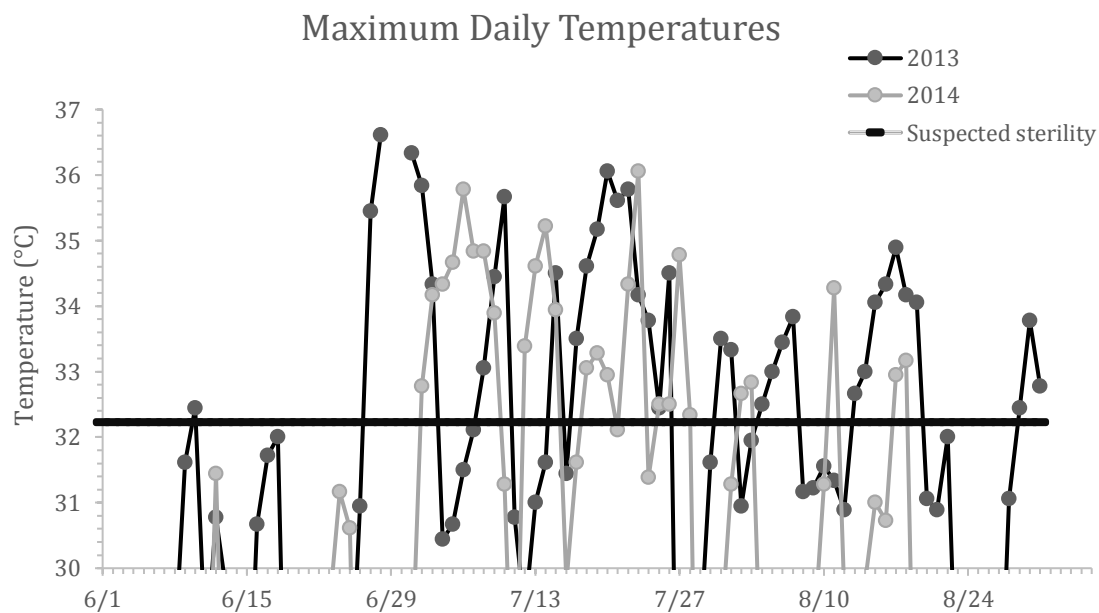
<sup>2</sup>K1 is located at UAES Kaysville in 2014



**Fig. 1** Irrigation and rainfall total for fields G1, G2 and K2 (panels A, B, and C, respectively). G1 is located at UAES Greenville in 2013; G2 is located at UAES Greenville in 2014, K1 is located at UAES Kaysville in 2014.



**Fig. 2** The timing of rain events and cumulative rain fall totals for each field during the period of beginning at planting (P) through establishment of line source irrigation treatment (L).



**Fig 3** Maximum daily temperatures during period of flowering and seed set in 2013 and 2014. Varieties included in this study have suspected pollen sterility above approximately 32 °C.



CHAPTER V: INTERACTIONS BETWEEN QUINOA (*CHENOPODIUM QUINOA*)  
AND THREE COMMON WEEDS IN A REPLACEMENT SERIES STUDY

Summary

Quinoa (*Chenopodium quinoa* Willd.) is an ancient crop with the potential to increase worldwide food security. Few growers within the US have experience with the crop but observations of competitive growth and tolerance to weed competition are widespread. With very limited herbicide options available and markets that favor organically grown quinoa, understanding the interactions between weeds and quinoa area essential to developing an effective cropping system. This study uses a replacement series design with quinoa and three common weed species: lambsquarters (*Chenopodium album* L.), red root pigweed (*Amaranthus retroflexus*) and green foxtail (*Setaria viridis*) and two fertility levels (60 and 240 mg N kg soil<sup>-1</sup>) repeated in two independent runs. Over most treatment and planting ratio combinations, quinoa had greater biomass accumulation than both red root pigweed and lambsquarters. Tissue nitrogen accumulation was similar between quinoa and two weed species, foxtail and red root pigweed, but lambsquarters had greater tissue nitrogen than quinoa. Green foxtail was the most competitive weed species although results varied between trial runs. Further research into the impact of emergence rate and planting density under field conditions is required for our region.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) has been cultivated for thousands of years in and around the Andes Mountains of South America in a variety of diverse ecosystems

(FAO, 2011; Sven-Erik Jacobsen 2003). Quinoa has received much attention in recent years from both the media and researchers as a potential crop to increase worldwide food stability due to its exceptional nutritional content and ability to grow in harsh climates (DePillis 2013; Romero and Shahriari 2011). Research and production of quinoa is now spreading worldwide and, as with many crop species, weed competition has been identified as a major source of yield loss in quinoa (Aguilar and Jacobsen 2003; Jacobsen et al. 2010). Not only does poor weed control limit total yield, but grain protein content has been shown to decrease from 17% to 12% when are not controlled (Jacobsen et al., 2010).

Quinoa is generally planted in the early spring and germinates rapidly. In the first few weeks after emergence, a critical window for weed growth, quinoa appears slow to increase above-ground biomass (Peterson and Murphy, 2015). These visual observations by growers and researchers have recently called into question quinoa's ability to out-compete weeds or companion crops such as undersown clover. In a recent field trial in Utah, quinoa appeared more competitive when fertilized with compost over non-amended quinoa, likely due to nutrient availability (unpublished). Currently, there are no studies that describe the relative competitive response of quinoa to weed species.

Weed management in a high value crop frequently involves the reliance on herbicides; however, use of herbicides in quinoa production is rare, as much of quinoa is produced organically and there are currently no herbicides labeled for quinoa in the United States (Jacobsen *et al.*, 2010). Therefore, weed control is a key issue in successful crop production, in both organic and conventional systems. Many potential quinoa

growers prefer to use organic management practices which offer the benefit of higher crop prices, reduced environmental impact and increased soil health. Organic farmers rely on multiple strategies to manage weeds including mechanical methods, planting date manipulation, alternative cropping schemes, or targeted nutrient applications (Bilalis *et al.*, 2014; Al-Khatib *et al.*, 1997). Jacobsen *et al.* (2010) report mechanical methods of weed control in organic quinoa production can be effective, but repeated cultivations are required and some damage to the quinoa crop itself is expected. Another and possibly complimentary approach may include optimizing nutrient additions to enhance crop growth while not stimulating growth of the predominant weed species.

Quinoa is considered to respond positively to increased soil nitrogen (N) fertility (Schulte auf'm Erley *et al.* 2005). However, increasing nutrient levels have also been shown to be beneficial to many weed species. For example, it has been demonstrated that response to N and phosphorus (P) fertilization levels are species dependent (Blackshaw *et al.*, 2009a; Blackshaw *et al.*, 2009b). Common lambsquarters (*Chenopodium album* L.), a close relative to quinoa, had a strong increase in biomass to N inputs and a moderate response to P (Blackshaw *et al.*, 2009a; Blackshaw *et al.*, 2009b). In a further study utilizing a replacement series design with various N levels and weed species, researchers concluded that tailoring nutrient inputs to crop/weed dynamics may be an effective method for controlling weeds and maintaining yields (Blackshaw and Brandt 2008).

Previous studies suggest a complex interaction between nutrient availability and competition between plants, which is highly species specific. By using a replacement series experimental design with three weed species of varying degrees of response to

nitrogen additions, the overall competitive response of quinoa to a range of growing conditions can be assessed. The goal of this study is to assess the relative competitive qualities of quinoa in relation to three common weed species, green foxtail (*Setaria viridis*), red root pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.), under high and low N levels. We hypothesized that quinoa will be more competitive at high N levels than low. We also hypothesize that quinoa will be more competitive than weed species with a reported low response to nutrient additions.

## Materials and methods

### Greenhouse design

The study is a modified replacement series design with quinoa as the crop, three weed species [green foxtail (*Setaria viridis*), redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.)] and two fertility levels (60 and 240 mg N kg soil<sup>-1</sup>). A total of eight plants per pot with proportions of 100:0, 75:25, 50:50, 25:75, and 0:100 (quinoa:weed, respectively) were used. The study included 4 replicates of each replacement series ratio and fertility level and was completed in two independent runs. The three species were selected due to their varied responses to N fertilizer (Blackshaw et al. 2009 a,b) and seed was purchased from HerbiSeed (United Kingdom). The quinoa variety was Oro de Valle (Washington State University), which is a variety developed for organic production in the Palouse region of eastern Washington state.

A soilless potting mix of 2:1:1 of peat moss, vermiculite, and perlite was mixed and brought to field capacity in 6 L pots. Two nitrogen (N) fertility rates were achieved with the use of a slow release fertilizer (Osmocote, Scotts Company, Marysville, OH). Micronutrients were applied uniformly to pots via split applications, regardless of N rate to provide adequate nutrients (Table 1). Pots were randomly placed within the greenhouse and rotated weekly. Both quinoa and a single weed species were over-seeded in pots and thinned to the assigned proportion within 1 week of emergence. Pots were watered with drip irrigation every other day to field capacity. Leachate was collected from random pots throughout the study to monitor N loss from the system at various plant ratio combinations. No differences were observed within fertility treatment levels and assumed to not be a factor in plant development. Greenhouse conditions maintained a 16h day length with maximum light intensity of 1,200 PPF and temperature range between 18 and 29 °C.

After 8 weeks, plants were harvested at ground level and sorted by species, and dried in a forced air oven at 60 °C. Dry samples were weighed, ground using a Wiley mill with a 40 mesh screen (Swedesboro, New Jersey) and processed for total N via combustion according to the manufacturer's protocol (Skalar Primacs Total Nitrogen Analyzer, Skalar Primac SLC Carbon Analyzer, respectively, Salt Lake City, Utah).

#### Statistical analyses

Plant species, fertilizer rate, and proportion of crop:weed comprised a three-way factorial in a CRD mixed model where pot was the experimental unit with plant species as repeated measures. The response variables of quinoa and weed biomass and total tissue

N were assessed as a proportion of the total pot population (Gealy *et al.*, 2005). Analyses of variance was accomplished with PROC GLIMMIX in the Statistical Analysis System for Windows version 9.4 (SAS Institute, Cary, NC). Variables were square-root or log transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. Multiple means comparisons were adjusted using the Tukey's method to control for family-wise Type I error rate. There were significant interactions between treatment combinations and trial runs, therefore, data from both runs were analyzed and are presented separately.

From the dry biomass weights and tissue N values obtained above, relative yield (RY) and aggressivity index (AI) values (Blackshaw and Brandt, 2008; Gealy *et al.*, 2005) of the weeds were calculated with the following equations:

$$RY = \frac{\text{Yield in mixture}}{\text{Yield in monoculture}}$$

$$AI = \frac{1}{2} * \left( \frac{\text{yield of weed species in mixture}}{\text{yield of weed species in monoculture}} - \frac{\text{yield of quinoa in mixture}}{\text{yield of quinoa in monoculture}} \right)$$

Relative yield and relative N uptake were calculated in order to produce replacement series diagrams (Blackshaw and Brandt, 2008; Gealy *et al.*, 2005). These diagrams allow for visual interpretation of the relative competitiveness of weed species in relation to quinoa. The AI serve as another method to measure the competitiveness of a weed species as compared with the quinoa (McGilchrist and Trenbath, 1971). Values less than zero indicate a species that is less competitive than quinoa, whereas positive AI values indicate a weed with greater competitive abilities than quinoa.

## Results

### Plant dry weights

#### Quinoa and foxtail

Total dry weight per pot as a function of the proportion of quinoa (Q) to weed was affected by several interactions for green foxtail (F). The three-way interaction of run\*proportion\*plant was significant at  $p=0.001$  (Table 2). In the run\*proportion\*plant interaction, the response was complex, with a clear difference between runs. In run 1, the interaction shows that while both species are equal in biomass accumulation in monoculture, quinoa was unaffected by the presence of F in proportions 3 and 2 while F biomass was greatly reduced in these proportions ( $p<0.001$  for all comparisons). Q had greater biomass than F ( $p<0.001$  for all comparisons) within run 1 in all proportions except for proportion 4, which did not differ. In contrast, in run 2, F in monoculture accumulated more biomass than Q in monoculture only ( $p<0.001$ ) while all other comparisons within proportions showed no differences in biomass. Run 2 was similar in that F in monoculture had the most biomass, proportion 3 was greater than 2 and 1, with no difference between proportions 2 and 1 ( $p<0.001$  for all significant comparisons except proportions 3 and 2 at  $p=0.002$ ); however, in run 2 for Q, proportion 4 was greater than 2 ( $p=0.003$ ), with 3 intermediate, all of which were greater than proportion 1 ( $p<0.001$  except proportion 1 and 2 at  $p<0.001$ ).

Run by fertility by proportion was also significant ( $p=0.004$ ) (Table 2). Within runs, plants receiving high fertility had greater biomass than low except proportion 1 in both runs and proportion 2 in run 1 which did not differ from each other ( $p<0.001$  except:

run 1 proportions 2 high and 4 low  $p=0.001$ ; proportions 3 high and 3 low  $p=0.018$ ; run 2 proportions 2 high and 2 low  $p=0.01$ ). Within the fertility treatments, a similar pattern was observed between runs. In the low fertility, proportion 4 was greater than all other proportions and proportion 3 was greater than proportion 1, with proportion 2 intermediate. In the high fertility, all proportions differed from each other within runs except proportions 1 and 2 during run 1, which did not differ (all comparisons  $p<0.001$  except run 1 and 2 proportion 2 high and 3 high at  $p=0.020$  and  $p=0.001$ , respectively). This complex interaction suggests a decreasing impact of N fertilization on plant yield as the number of plants per species decreases.

The interaction of fertility and plant ( $p<0.001$ ) showed that both Q and F had higher biomass yields with high fertility over low (Table 2). At the same time within both fertility treatments, F dry weights were lower than Q averaged over all runs and proportions (all comparisons  $p<0.001$ , except low F and low Q differ at  $p=0.045$ ).

#### Quinoa and lambsquarters

Plant species interactions with proportions were significant ( $p<0.001$ ) (Table 3). All lambsquarters (L) grown in mixture with Q had lower dry weights than any Q proportion ( $p<0.001$  for all comparisons). Q proportions did not differ from each other, and L grown in monoculture differed from only quinoa proportions 4 and 2 ( $p=0.0023$  and  $p=0.026$ , respectively). Lambsquarters in proportion 4 had greater biomass than any other L proportion ( $p<0.001$  for all comparisons), followed by proportion 3 which was greater than proportion 1 ( $p<0.001$ ), with proportion 2 intermediate. This suggests that



the change in plant populations did not affect Q biomass but did decrease the growth of L with Q presence increased.

Fertility rate also had an impact on Q but not L yield (Table 3). The interaction of fertility by plant by run was significant ( $p=0.002$ ). Quinoa receiving high fertility had greater biomass than low fertility quinoa, both of which were greater than any lambsquarters/fertility combination.

#### Quinoa and redroot pigweed

The yield of redroot pigweed (R) grown in mixture with quinoa was markedly reduced ( $p<0.001$ ) from the monoculture yield in both fertility levels, as demonstrated by the significant fertility by proportion by plant interaction (Table 4). In the high fertility, Q had greater yield than R ( $p<0.001$  for all comparisons) in all proportions except 4, in which there was no difference between Q and R. In the low fertility, quinoa was greater than R for within each proportion ( $p<0.001$  for all comparisons except proportion 4 Q differs from R  $p<0.001$ ). Quinoa high fertility was greater than Q low in each proportion ( $p<0.001$  for proportion 1;  $p<0.001$  for proportions 2 and 3;  $p<0.001$  for proportion 4) while R high was greater than R low in only proportion 4 ( $p<0.001$ ). This suggests the increased presence of quinoa was the driving factor in R biomass accumulation. While the run by plant interaction was also significant due to different weights between runs ( $p<0.001$ ), the trend remained constant with quinoa having higher biomass than redroot pigweed in each run. The proportion by fertility interaction ( $p=0.006$ ) revealed high fertility had more biomass than the low proportion averaged over all plants and runs.

### Total N assimilation

The interaction of fertility and plant was significant ( $p=0.007$ ) for Q:L (Table 5). Lambsquarters had greater N accumulation than quinoa within each fertilizer level. Averaged over both runs and proportions, lambsquarters with high fertility had greatest total N followed by low N lambsquarters and high quinoa, which did not differ from each other, with low N quinoa having the lowest tissue N ( $p<0.001$  for all comparisons). The interaction of plant and proportion was significant for Q:L ( $p<0.001$ ). Averaged over fertility levels and runs, lambsquarters had higher N content than all quinoa ratios (all comparisons  $p<0.001$  except proportion 1 Q differs from proportion 2 L at  $p=0.005$ ). Within lambsquarters, proportion 4 had higher N than any other proportion ( $p=0.004$ ,  $p<0.001$ , and  $p<0.001$  for comparisons with proportions 1, 2, and 3, respectively).

In the Q:F mixtures, fertility was a significant main effect ( $p<0.001$ , respectively) with high fertility having greater tissue N than low, averaged over all runs, plants and proportions (Table 6). There was a significant interaction of run and plant ( $p<0.001$ ) in which in run 1, green foxtail had higher tissue N than quinoa ( $p=0.012$ ) yet in run 2, quinoa had higher tissue N than green foxtail ( $p<0.001$ ). A three-way interaction of run, proportion and plant ( $p=0.013$ ) showed that during run 1, there were no differences in tissue N content within or between each plant species; however, in run 2, F proportion 4 was less than F proportion 1 ( $p=0.011$ ) and Q proportions 3 ( $p=0.008$ ) and 4 ( $p<0.001$ ), with no other differences noted.

In the Q:R mixtures, averaged over both plants and proportions, the fertility by run interaction was significant ( $p<0.001$ ). The highest N accumulation was in high

fertility plants in run 2, run 2 low fertility and run 1 high fertility, which did not differ from each other. Low fertility treated plants in run1 had the lowest tissue N (Table 7). The proportion by plant and run by proportion by plant interactions were also significant ( $p < 0.001$  and  $p = 0.017$ , respectively). Within each proportion, quinoa in run 2 had higher total N than in run 1, except in proportion 2 where there was no difference (proportion 1  $p = 0.001$ , proportion 3  $p < 0.001$ , proportion 4  $p = 0.005$ ). The total N content of R did not differ between runs except in proportions 1 and 4, which had greater N in run 2 than run 1 ( $p = 0.019$  and  $p = 0.021$ , respectively). During each independent run, R had greater total N in proportion 4 than quinoa in proportion 4, but no other proportions differed within run (run 1  $p = 0.001$ , run 2  $p = 0.002$ ).

Averaged over all levels of fertility, plant and proportions, tissue N was greater in the second run over the first run for each quinoa/weed mixtures ( $p < 0.001$  for Q:L and Q:R,  $p = 0.002$  for Q:F).

#### Replacement series diagrams and aggressivity indices

Replacement series diagrams show the interaction of quinoa and weed species with reference to projected yields that change linearly with ratio. If the RY of a species falls below the projected line, it is interpreted as a lack of competitive ability against the other species; similarly, RY above the projected line represents a competitive advantage. There was a significant effect ( $p < 0.001$ ) of trial run on total dry weight of quinoa:foxtail mixtures and therefore the replacement series diagrams were separated by run (Table 2 and Figure 1). Quinoa had higher RY than projected in run 1 at both low and high N rates (Figure 1 A and 1C, respectively) while in run 2, both species were more closely fit to the

projected RYs (Figure 1B and 1D). This suggests quinoa and green foxtail competed equally for available resources in the second trial. While the results are different between trial runs, green foxtail was the closest to projected RY of any of the weed species, which suggests it may have the most impact on quinoa's resource allocation.

Replacement series diagrams for Q:R and Q:L were similar (Figures 2 and 3, respectively). Quinoa was much higher than projected RY in all runs and nitrogen levels, while red root pigweed or lambsquarters was much lower than projected. These diagrams indicate an overwhelming yield advantage of quinoa over both lambsquarters and redroot pigweed, regardless of planting ratio.

Replacement series diagrams for relative N (RN) assimilation compares the N uptake of a species in monoculture versus mixed plantings. In this study, the response of quinoa tissue N to planting proportions varied with weed species. When quinoa and green foxtail interacted, quinoa had a near steady RN over the range of proportions (Figure 4). Except in the high fertility during run 2 where quinoa's RN values increased with increasing quinoa presence (Figure 4D), the RN of quinoa held near 1 regardless of other plant proportions. Relative N of foxtail plants in run 2, both high and low fertility levels, increased with increasing quinoa presence, perhaps suggesting the weed species has an enhanced assimilation ability when grown in competition (Figure 4 B and D). Conversely, when quinoa was grown with lambsquarters or redroot pigweed, the RN of quinoa was higher in mixed culture pots than in monoculture (Figures 5 and 6). Both lambsquarters and redroot pigweed had lower RN accumulations with increasing presence of quinoa (Figures 5 and 6).

Aggressivity indices (AI) were calculated for both yield and N assimilation. An AI value less than zero has been interpreted as a species that is less competitive than quinoa, whereas positive AI values indicate a weed with greater competitive abilities than quinoa. In the yield AI comparisons, both lambsquarters and red root pigweed were consistently negative at all planting ratios (Table 8). Green foxtail was also negative except for in run 2 at the 25:75 ratio (Q:F), where green foxtail was more competitive than quinoa. These are consistent with the dry weight and total N assimilation trends, suggesting at higher planting numbers, green foxtail was the most competitive with quinoa while lambsquarters and red root had very limited impact on quinoa growth. Tissue nitrogen AI values were highly variable and generally close to 0, suggesting similar N assimilation in mixed plantings of both weeds and quinoa (Table 9).

## Discussion

In the pairings between both R and L with Q, quinoa had higher biomass and tissue N than either weed species. The effects of increased weed presence on quinoa's growth and N assimilation were minimal with either of weed competitor. The interactions between F and Q were more complicated and varied by trial run. In the first trial run of Q and F pairings, the increased presence of Q yielded a decrease in F biomass. However, in the second trial run, the means of the monocultures differed between Q and F, but the mixed plantings did not.

Quinoa was fast to germinate and establish in all treatment and ratio combinations. This likely led to higher relative yield values. Both L and R took longer to germinate and emerge than quinoa, which may have been a critical advantage to quinoa's

growth and resource acquisition. On average, quinoa germinated and emerged in less than 7 days, similar to F. Red root pigweed and lambsquarters lagged by 3-5 days. Field observations show quinoa rapidly emerges and develops 2-4 true leaves followed by a lag in above ground growth, presumably when rapid growth of the tap root system takes priority. This lag in above ground growth slows canopy closure and allows early season weed competitors, like green foxtail, a window of advantage. In a separate study conducted as an organic field trial, green foxtail proved the most competitive as it emerged early in the season in a similar rate to quinoa and was particularly pronounced under lower N and P fertility treatments (unpublished, Utah State University).

The replacement series diagrams depict the RY advantage of quinoa in most pairings. This suggests quinoa was able to access more resources and develop quicker than lambsquarters or red root pigweed. As with cultivated plant species, weed species respond to nutrient availability in a variety of ways. Some weeds are considered luxury nutrient users and increase growth proportional to available nutrients whereas other species reach their maximum growth rates with very little nutrient inputs. Blackshaw *et al.* (2003; 2008) determined the response of several weed species to both N and P fertilizer inputs. Blackshaw *et al.* (2003) reported lambsquarters and redroot pigweed N have a consistent N assimilation rate regardless of N available while green foxtail assimilation percentage decreased with N application rate. Our results demonstrate that lambsquarters N uptake was greater than quinoa, while quinoa and red root pigweed are similar. The tissue N in quinoa:foxtail mixtures was variable, in similar fashion as yield, with no differences noted in run 1 but higher N uptake in run 2 in Q monoculture over F

monoculture. Knowledge of the relative competitive abilities of quinoa will allow growers to better target nutrient inputs that will favor the crop instead of the prevalent weed species.

Weed control in quinoa has been shown to improve yields and grain protein content, yet limited options exist without the use of labeled herbicides or in organic systems. Mechanical cultivation has been shown to reduce weed biomass by between 40-70%, but also causes damage to the quinoa plants (Jacobsen *et al.*, 2010). In the same study by Jacobsen *et al.* (2010), a false seed bed which required a 2-week delay in seeding, followed by repeated harrowing, resulted in yield loss, suspected to be from the delay in seeding dates only. Quinoa needs to be seeded early to be most productive. Currently there are no published studies that have examined the impact of alternate cropping systems on weed control in quinoa however, intercropping or relay cropping may provide weed suppression. Further research into cropping systems and management strategies to reduce weed pressure in quinoa are essential.

Interactions between crops and weeds are highly dependent on the density of species populations. In replicated greenhouse studies, there are generally three distinct designs to examine the interactions of weeds and crops: pair-wise, replacement or additive models (Gibson *et al.*, 1999; Rejmánek *et al.*, 1989; Snaydon, 1991). Pair-wise designs use a fixed ratio of two species, typically 1:1, whereas replacement and additive models take different approaches to how the species density is varied (Gibson *et al.*, 1999). In a replacement series model, the total plant population is held constant and the ratio of crop to weed species is varied at a predictable rate while an additive model varies

the total density of plants, generally holding the crop density constant and varying the weed species density (Rejmánek *et al.*, 1989). The replacement series model ranges from exclusively crop treatments to exclusively weed treatments with intermediary ratios of species in an attempt to quantify relative competitive qualities of a crop (Rejmánek *et al.*, 1989). Each of these experimental designs have noted flaws in their ability to predict inter- and intra-specific competition at the field scale level. While the replacement series design has a limited ability to isolate inter- and intra-specific competitive effects, this study does provide a first look at interactions between quinoa and common weed species of the region as a basis for further research.

## Conclusion

Quinoa responded to increased nitrogen with increased biomass and higher tissue N. The effect of weed presence on the growth and N content of quinoa was similar between treatments with redroot pigweed and lambsquarters. In general, quinoa was unaffected by the increase in weed presence at different planting ratios. Both biomass yield and tissue N assimilation suggest green foxtail is the most competitive with quinoa of the weeds tested in this study. The slower emergence rate of redroot pigweed and lambsquarters likely provided an advantage to quinoa; conversely, the rapid establishment of green foxtail may have been the reason for greater impact on growth, which was variable between trial runs. Further study under field conditions with various fertility levels and cropping system management strategies are needed to fully describe the competitive abilities of quinoa.



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Tables and figures

**Table 1** Nutrient inputs for both trial runs applied as a combination of extended release fertilizer and split applications of micro-nutrient formulations.

| <b>Nutrient</b> | <b>Low<br/>Rate<br/>mg/kg</b> | <b>High<br/>Rate<br/>mg/kg</b> |
|-----------------|-------------------------------|--------------------------------|
| N               | 60                            | 240                            |
| P               | 77                            | 307                            |
| K               | 293                           | 1170                           |
| S               | 25                            | 25                             |
| Zn              | 0.35                          | 0.35                           |
| Cu              | 0.16                          | 0.16                           |
| Mn              | 1.07                          | 1.07                           |
| Mo              | 0                             | 0                              |
| B               | 0                             | 0                              |
| Fe              | 2.84                          | 2.84                           |
| Mg              | 4.42                          | 4.42                           |

**Table 2** Means of dry weights per pot of quinoa and foxtail species. Letters signify statistically significant differences at  $p < 0.05$ .

|                                 |        | <b>Species mixture</b>  |                     |                     |                     |
|---------------------------------|--------|-------------------------|---------------------|---------------------|---------------------|
|                                 |        | <b>Quinoa : Foxtail</b> |                     |                     |                     |
| <b>Fertility*plant</b>          | Low    | <b>Quinoa</b>           |                     | <b>Foxtail</b>      |                     |
|                                 | High   | 12.9 <sup>C</sup>       | 12.1 <sup>D</sup>   | 24.5 <sup>A</sup>   | 18.2 <sup>B</sup>   |
| <b>Run*fertility</b>            | Low    | <b>Run 1</b>            |                     | <b>Run 2</b>        |                     |
|                                 | High   | 12.3 <sup>C</sup>       | 12.7 <sup>C</sup>   | 19.6 <sup>B</sup>   | 23.1 <sup>A</sup>   |
| <b>Run*plant</b>                | Quinoa | <b>Run 1</b>            |                     | <b>Run 2</b>        |                     |
|                                 | Weed   | 21.6 <sup>A</sup>       | 15.8 <sup>C</sup>   | 10.3 <sup>D</sup>   | 20.0 <sup>B</sup>   |
| <b>Run*proportion*plant</b>     |        | <b>Quinoa</b>           |                     | <b>Foxtail</b>      |                     |
|                                 |        | <b>Run 1</b>            | <b>Run 2</b>        | <b>Run 1</b>        | <b>Run 2</b>        |
|                                 | 1      | 16.8 <sup>CD</sup>      | 7.49 <sup>F</sup>   | 1.96 <sup>G</sup>   | 8.73 <sup>EF</sup>  |
|                                 | 2      | 21.8 <sup>BC</sup>      | 15.2 <sup>D</sup>   | 3.64 <sup>G</sup>   | 12.8 <sup>DE</sup>  |
|                                 | 3      | 21.9 <sup>BC</sup>      | 18.6 <sup>CD</sup>  | 8.96 <sup>EF</sup>  | 21.1 <sup>BC</sup>  |
|                                 | 4      | 26.0 <sup>B</sup>       | 22.0 <sup>BC</sup>  | 26.6 <sup>B</sup>   | 37.3 <sup>A</sup>   |
| <b>Run*fertility*proportion</b> |        | <b>Low</b>              |                     | <b>High</b>         |                     |
|                                 |        | <b>Run 1</b>            | <b>Run 2</b>        | <b>Run 1</b>        | <b>Run 2</b>        |
|                                 | 1      | 14.2 <sup>G</sup>       | 7.45 <sup>FG</sup>  | 10.8 <sup>EFG</sup> | 8.77 <sup>EFG</sup> |
|                                 | 2      | 10.9 <sup>EFG</sup>     | 9.29 <sup>EFG</sup> | 14.5 <sup>E</sup>   | 18.7 <sup>D</sup>   |
|                                 | 3      | 12.0 <sup>EF</sup>      | 12.3 <sup>E</sup>   | 18.9 <sup>D</sup>   | 27.5 <sup>BC</sup>  |
|                                 | 4      | 19.7 <sup>D</sup>       | 21.6 <sup>CD</sup>  | 32.9 <sup>AB</sup>  | 37.6 <sup>A</sup>   |
| <b>ANOVA p-values</b>           |        |                         |                     |                     |                     |
| Fertility*plant                 |        | <0.001                  |                     |                     |                     |
| Run*fertility                   |        | 0.014                   |                     |                     |                     |
| Run*plant                       |        | <0.001                  |                     |                     |                     |
| Run*fertility*proportion        |        | 0.004                   |                     |                     |                     |
| Run*proportion*plant            |        | <0.001                  |                     |                     |                     |

**Table 3** Means of dry weights per pot of quinoa and lambsquarters species. Letters signify statistically significant differences at  $p < 0.05$ .

| <b>Species mixture</b>        |                    |                   |                      |                   |  |
|-------------------------------|--------------------|-------------------|----------------------|-------------------|--|
| <b>Quinoa : Lambsquarters</b> |                    |                   |                      |                   |  |
| <b>Proportion*plant</b>       | <b>Quinoa</b>      |                   | <b>Lambsquarters</b> |                   |  |
| 1                             | 22.0 <sup>AB</sup> |                   | 0.72 <sup>D</sup>    |                   |  |
| 2                             | 24.3 <sup>A</sup>  |                   | 1.50 <sup>CD</sup>   |                   |  |
| 3                             | 23.5 <sup>AB</sup> |                   | 2.43 <sup>C</sup>    |                   |  |
| 4                             | 26.0 <sup>A</sup>  |                   | 18.6 <sup>B</sup>    |                   |  |
| <b>Run*fertility*plant</b>    | <b>Quinoa</b>      |                   | <b>Lambsquarters</b> |                   |  |
|                               | <b>Run 1</b>       | <b>Run 2</b>      | <b>Run 1</b>         | <b>Run 2</b>      |  |
| Low                           | 20.2 <sup>B</sup>  | 14.2 <sup>B</sup> | 4.04 <sup>C</sup>    | 5.41 <sup>C</sup> |  |
| High                          | 29.3 <sup>A</sup>  | 31.6 <sup>A</sup> | 8.50 <sup>C</sup>    | 4.44 <sup>C</sup> |  |
| <b>ANOVA p-values</b>         |                    |                   |                      |                   |  |
| Fertility*plant               |                    | <0.001            |                      |                   |  |
| Proportion*plant              |                    | <0.001            |                      |                   |  |
| Run*fertility*plant           |                    | 0.002             |                      |                   |  |

**Table 4** Means of dry weights per pot of quinoa and redroot pigweed species. Letters signify statistically significant differences at  $p < 0.05$ .

|                                   |        | <b>Species mixture</b>          |                        |                        |                        |
|-----------------------------------|--------|---------------------------------|------------------------|------------------------|------------------------|
|                                   |        | <b>Quinoa : Redroot pigweed</b> |                        |                        |                        |
| <b>Fertility</b>                  |        |                                 |                        |                        |                        |
|                                   | Low    |                                 |                        | 9.54 <sup>B</sup>      |                        |
|                                   | High   |                                 |                        | 18.8 <sup>A</sup>      |                        |
| <b>Plant</b>                      |        | <b>Run 1</b>                    |                        | <b>Run 2</b>           |                        |
|                                   | Quinoa | 23.5 <sup>A</sup>               |                        | 19.7 <sup>B</sup>      |                        |
|                                   | Weed   | 5.82 <sup>D</sup>               |                        | 7.67 <sup>C</sup>      |                        |
| <b>Proportion*plant</b>           |        | <b>Quinoa</b>                   |                        | <b>Redroot pigweed</b> |                        |
|                                   | 1      | 17.2 <sup>B</sup>               |                        | 1.15 <sup>D</sup>      |                        |
|                                   | 2      | 22.3 <sup>A</sup>               |                        | 1.92 <sup>D</sup>      |                        |
|                                   | 3      | 21.6 <sup>AB</sup>              |                        | 5.13 <sup>C</sup>      |                        |
|                                   | 4      | 25.5 <sup>A</sup>               |                        | 18.8 <sup>B</sup>      |                        |
| <b>Fertility*proportion*plant</b> |        | <b>Low</b>                      |                        | <b>High</b>            |                        |
|                                   |        | <b>Quinoa</b>                   | <b>Redroot pigweed</b> | <b>Quinoa</b>          | <b>Redroot pigweed</b> |
|                                   | 1      | 12.2 <sup>E</sup>               | 0.98 <sup>H</sup>      | 22.2 <sup>BC</sup>     | 1.33 <sup>H</sup>      |
|                                   | 2      | 14.2 <sup>DE</sup>              | 1.49 <sup>H</sup>      | 31.6 <sup>A</sup>      | 2.35 <sup>GH</sup>     |
|                                   | 3      | 13.8 <sup>DE</sup>              | 4.13 <sup>FG</sup>     | 29.4 <sup>AB</sup>     | 6.13 <sup>F</sup>      |
|                                   | 4      | 19.0 <sup>CD</sup>              | 10.5 <sup>E</sup>      | 32.0 <sup>A</sup>      | 27.1 <sup>AB</sup>     |
| <b>ANOVA p-values</b>             |        |                                 |                        |                        |                        |
| Fertility                         |        | <0.001                          |                        |                        |                        |
| Plant                             |        | <0.001                          |                        |                        |                        |
| Proportion                        |        | <0.001                          |                        |                        |                        |
| Run*plant                         |        | <0.001                          |                        |                        |                        |
| Proportion*plant                  |        | <0.001                          |                        |                        |                        |
| Fertility*proportion*plant        |        | <0.001                          |                        |                        |                        |

**Table 5** Means of dry tissue nitrogen (N) of quinoa and lambsquarters species. Letters signify statistically significant differences at  $p < 0.05$ .

| <b>Species mixture</b>        |        |                      |                             |
|-------------------------------|--------|----------------------|-----------------------------|
| <b>Quinoa : Lambsquarters</b> |        |                      |                             |
| <b>Run</b>                    | First  |                      | 2.38 <sup>B</sup>           |
|                               | Second |                      | 3.03 <sup>A</sup>           |
| <b>Fertility*plant</b>        |        | <b><u>Quinoa</u></b> | <b><u>Lambsquarters</u></b> |
|                               | Low    | 1.87 <sup>C</sup>    | 2.56 <sup>B</sup>           |
|                               | High   | 2.55 <sup>B</sup>    | 3.89 <sup>A</sup>           |
| <b>Plant</b>                  | Quinoa |                      | 2.21 <sup>B</sup>           |
|                               | Weed   |                      | 3.22 <sup>A</sup>           |
| <b>Proportion</b>             | 1      |                      | 2.70 <sup>AB</sup>          |
|                               | 2      |                      | 2.54 <sup>B</sup>           |
|                               | 3      |                      | 2.55 <sup>B</sup>           |
|                               | 4      |                      | 3.01 <sup>A</sup>           |
| <b>Proportion*plant</b>       |        | <b><u>Quinoa</u></b> | <b><u>Lambsquarters</u></b> |
|                               | 1      | 2.28 <sup>C</sup>    | 3.12 <sup>B</sup>           |
|                               | 2      | 2.22 <sup>C</sup>    | 2.87 <sup>B</sup>           |
|                               | 3      | 2.14 <sup>C</sup>    | 2.96 <sup>B</sup>           |
|                               | 4      | 2.12 <sup>C</sup>    | 2.97 <sup>A</sup>           |
| <b>ANOVA p-values</b>         |        |                      |                             |
| Run                           |        |                      | <0.001                      |
| Proportion                    |        |                      | 0.004                       |
| Fertility*plant               |        |                      | 0.007                       |
| Proportion*plant              |        |                      | <0.001                      |

**Table 6** Means of dry tissue nitrogen (N) of quinoa and foxtail species. Letters signify statistically significant differences at  $p < 0.05$ .

|                             |                                | <b>Species mixture</b> |                      |                      |                      |
|-----------------------------|--------------------------------|------------------------|----------------------|----------------------|----------------------|
|                             |                                | <b>Quinoa:Foxtail</b>  |                      |                      |                      |
| <b>Run</b>                  | First                          | 1.88 <sup>B</sup>      |                      |                      |                      |
|                             | Second                         | 2.10 <sup>A</sup>      |                      |                      |                      |
| <b>Fertility</b>            | Low                            | 1.69 <sup>B</sup>      |                      |                      |                      |
|                             | High                           | 2.27 <sup>A</sup>      |                      |                      |                      |
| <b>Run*plant</b>            |                                | <b>Run 1</b>           |                      | <b>Run 2</b>         |                      |
|                             | Quinoa                         | 1.77 <sup>C</sup>      |                      | 2.28 <sup>A</sup>    |                      |
|                             | Foxtail                        | 2.00 <sup>B</sup>      |                      | 1.93 <sup>BC</sup>   |                      |
| <b>Run*proportion*plant</b> |                                | <b>Quinoa</b>          |                      | <b>Foxtail</b>       |                      |
|                             |                                | <b>Run 1</b>           | <b>Run 2</b>         | <b>Run 1</b>         | <b>Run 2</b>         |
|                             | 1                              | 1.69 <sup>CD</sup>     | 2.12 <sup>ABDC</sup> | 2.11 <sup>ABCD</sup> | 2.24 <sup>ABC</sup>  |
|                             | 2                              | 1.89 <sup>BCD</sup>    | 2.14 <sup>ABCD</sup> | 2.09 <sup>ABCD</sup> | 2.05 <sup>ABCD</sup> |
|                             | 3                              | 1.71 <sup>BCD</sup>    | 2.23 <sup>AB</sup>   | 1.79 <sup>BCD</sup>  | 1.85 <sup>BCD</sup>  |
|                             | 4                              | 1.79 <sup>BCD</sup>    | 2.56 <sup>A</sup>    | 2.03 <sup>ABCD</sup> | 1.55 <sup>D</sup>    |
| <b>ANOVA p-values</b>       |                                |                        |                      |                      |                      |
|                             | Run                            | 0.002                  |                      |                      |                      |
|                             | Fertility                      | <0.001                 |                      |                      |                      |
|                             | Run*plant                      | <0.001                 |                      |                      |                      |
|                             | Run*proportion*plant           | 0.013                  |                      |                      |                      |
|                             | Run*fertility*proportion*plant | 0.044                  |                      |                      |                      |



**Table 7** Means of dry tissue nitrogen (N) of quinoa and redroot pigweed species. Letters signify statistically significant differences at  $p < 0.05$ .

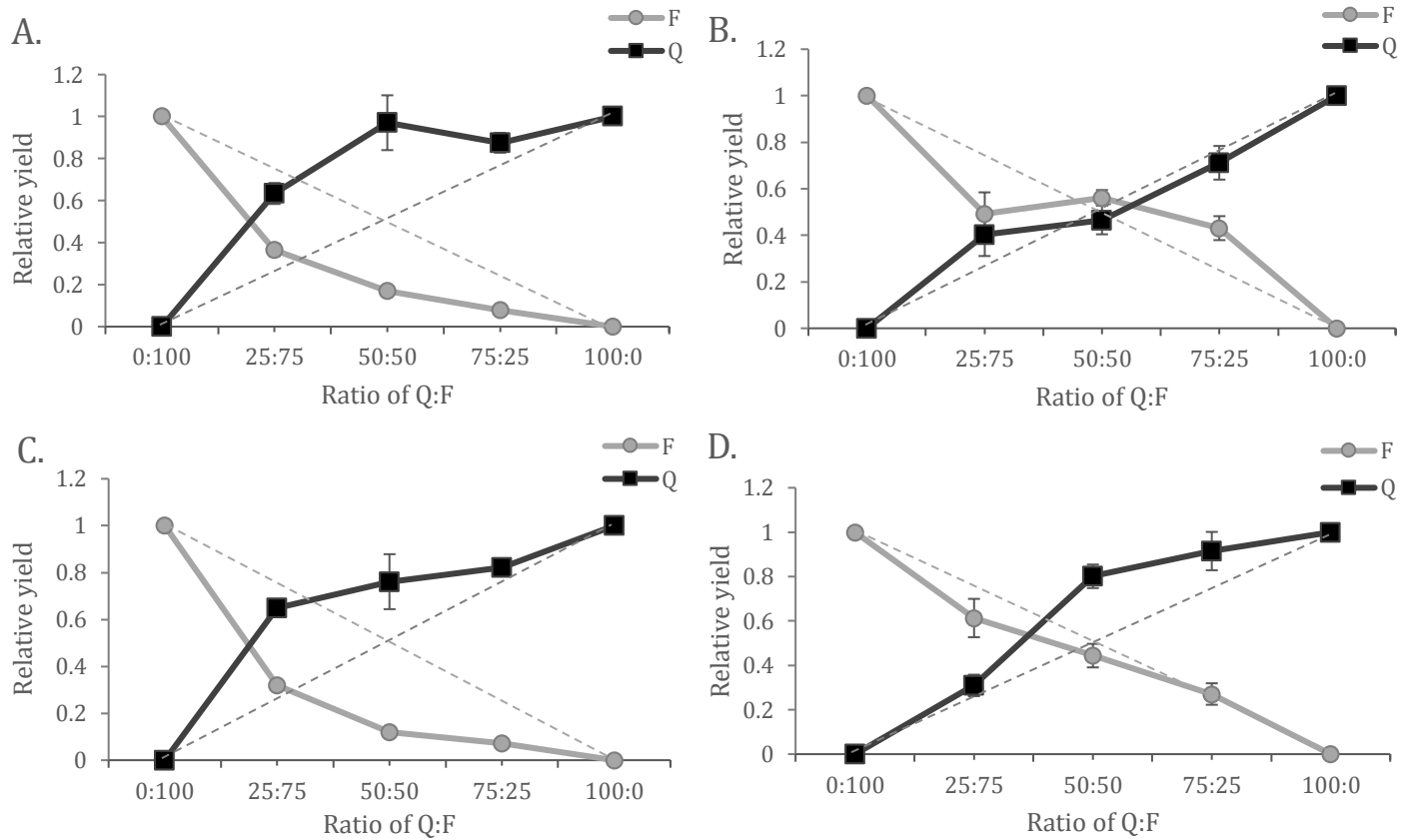
| <b>Species mixture</b>          |                       |                       |                        |                       |
|---------------------------------|-----------------------|-----------------------|------------------------|-----------------------|
| <b>Quinoa : Redroot pigweed</b> |                       |                       |                        |                       |
| <b>Run*fertility</b>            | <b>Run 1</b>          |                       | <b>Run 2</b>           |                       |
| Low                             | 1.82                  |                       | 2.20                   |                       |
| High                            | 2.23                  |                       | 3.13                   |                       |
| <b>Proportion*plant</b>         | <b>Quinoa</b>         |                       | <b>Redroot pigweed</b> |                       |
| 1                               | 2.44 <sup>B</sup>     |                       | 2.20 <sup>B</sup>      |                       |
| 2                               | 2.20 <sup>B</sup>     |                       | 2.24 <sup>B</sup>      |                       |
| 3                               | 2.40 <sup>B</sup>     |                       | 2.54 <sup>B</sup>      |                       |
| 4                               | 2.10 <sup>B</sup>     |                       | 2.90 <sup>A</sup>      |                       |
| <b>Run*proportion*plant</b>     | <b>Quinoa</b>         |                       | <b>Redroot pigweed</b> |                       |
|                                 | <b>Run 1</b>          | <b>Run 2</b>          | <b>Run 1</b>           | <b>Run 2</b>          |
| 1                               | 2.02 <sup>CDEFG</sup> | 2.86 <sup>AB</sup>    | 1.88 <sup>EFG</sup>    | 2.52 <sup>BCD</sup>   |
| 2                               | 2.03 <sup>CDEFG</sup> | 2.37 <sup>BCDEF</sup> | 1.90 <sup>DEFG</sup>   | 2.58 <sup>BCDE</sup>  |
| 3                               | 1.87 <sup>FG</sup>    | 2.93 <sup>AB</sup>    | 2.19 <sup>CDEFG</sup>  | 2.33 <sup>BCDEF</sup> |
| 4                               | 1.75 <sup>G</sup>     | 2.44 <sup>BCDEF</sup> | 2.54 <sup>BC</sup>     | 3.26 <sup>A</sup>     |
| <b>ANOVA p-values</b>           |                       |                       |                        |                       |
| Run*fertility                   | <0.001                |                       |                        |                       |
| Proportion*plant                | <0.001                |                       |                        |                       |
| Run*proportion*plant            | 0.017                 |                       |                        |                       |

**Table 8** Yield aggressivity indices (AI) separated by fertility level and weed ratio and trial run for foxtail.

|                                 | Quinoa:<br>Lambsquarters | Species Mixture    |        | Quinoa:<br>Redroot<br>pigweed |
|---------------------------------|--------------------------|--------------------|--------|-------------------------------|
|                                 |                          | Quinoa:<br>Foxtail |        |                               |
| Planting Ratio<br>(Quinoa:Weed) |                          | Run 1              | Run 2  |                               |
| <b>Low Nitrogen</b>             |                          |                    |        |                               |
| 25:75                           | -0.269                   | -0.134             | 0.045  | -0.125                        |
| 50:50                           | -0.024                   | -0.062             | -0.015 | -0.013                        |
| 75:25                           | -0.443                   | -0.398             | -0.141 | -0.345                        |
| <b>High Nitrogen</b>            |                          |                    |        |                               |
| 25:75                           | -0.372                   | -0.165             | 0.152  | -0.213                        |
| 50:50                           | -0.018                   | -0.049             | -0.014 | -0.037                        |
| 75:25                           | -0.250                   | -0.375             | -0.322 | -0.258                        |

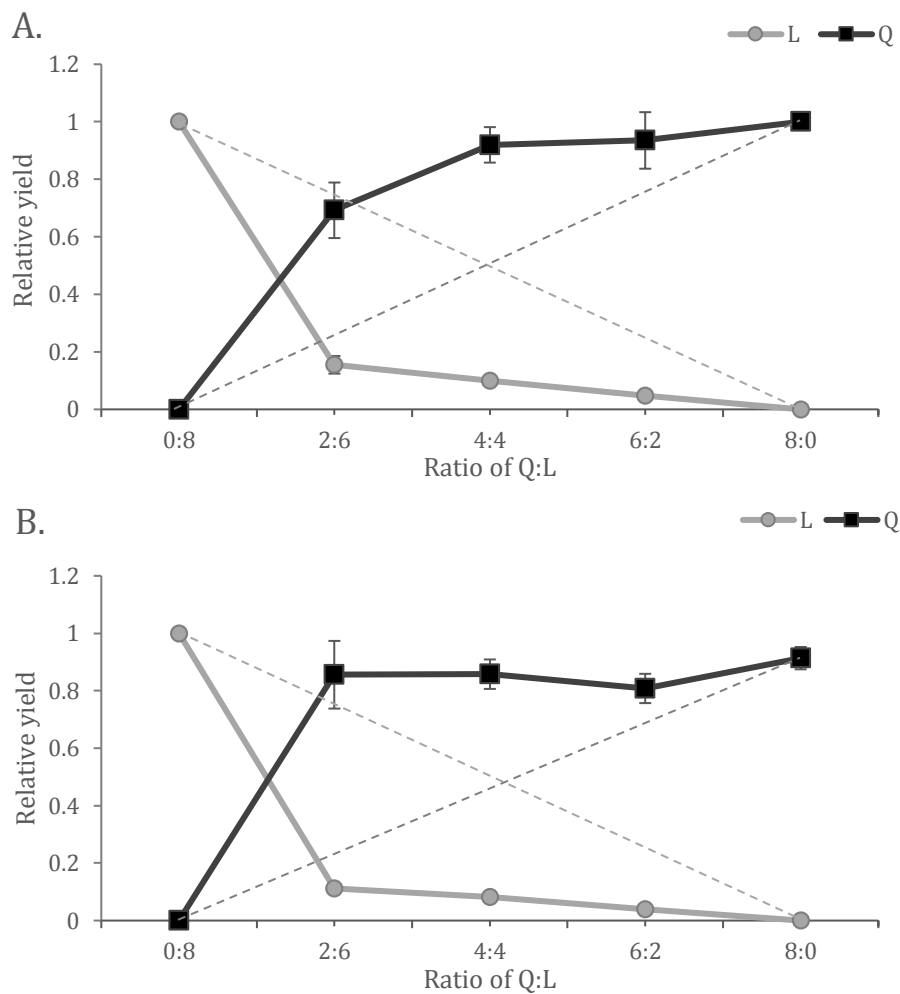
**Table 9** Nitrogen aggressivity indices (AI) values separated by run and fertility level for each crop species/ratio combination.

| Planting Ratio<br>(Quinoa:Weed) | Species Mixtures         |        |                    |        |                            |        |
|---------------------------------|--------------------------|--------|--------------------|--------|----------------------------|--------|
|                                 | Quinoa:<br>Lambsquarters |        | Quinoa:<br>Foxtail |        | Quinoa:<br>Redroot pigweed |        |
|                                 | Run 1                    | Run 2  | Run 1              | Run 2  | Run 1                      | Run 2  |
| <b>Low Nitrogen</b>             |                          |        |                    |        |                            |        |
| 25:75                           | -0.021                   | 0.012  | -0.019             | -0.020 | -0.015                     | 0.042  |
| 50:50                           | 0.015                    | 0.030  | -0.011             | 0.010  | -0.006                     | 0.014  |
| 75:25                           | 0.124                    | 0.036  | -0.025             | 0.061  | 0.016                      | -0.014 |
| <b>High Nitrogen</b>            |                          |        |                    |        |                            |        |
| 25:75                           | 0.001                    | -0.013 | -0.001             | -0.048 | 0.016                      | 0.062  |
| 50:50                           | 0.015                    | -0.020 | -0.016             | 0.034  | 0.052                      | -0.009 |
| 75:25                           | -0.029                   | 0.009  | 0.008              | -0.008 | -0.023                     | 0.009  |



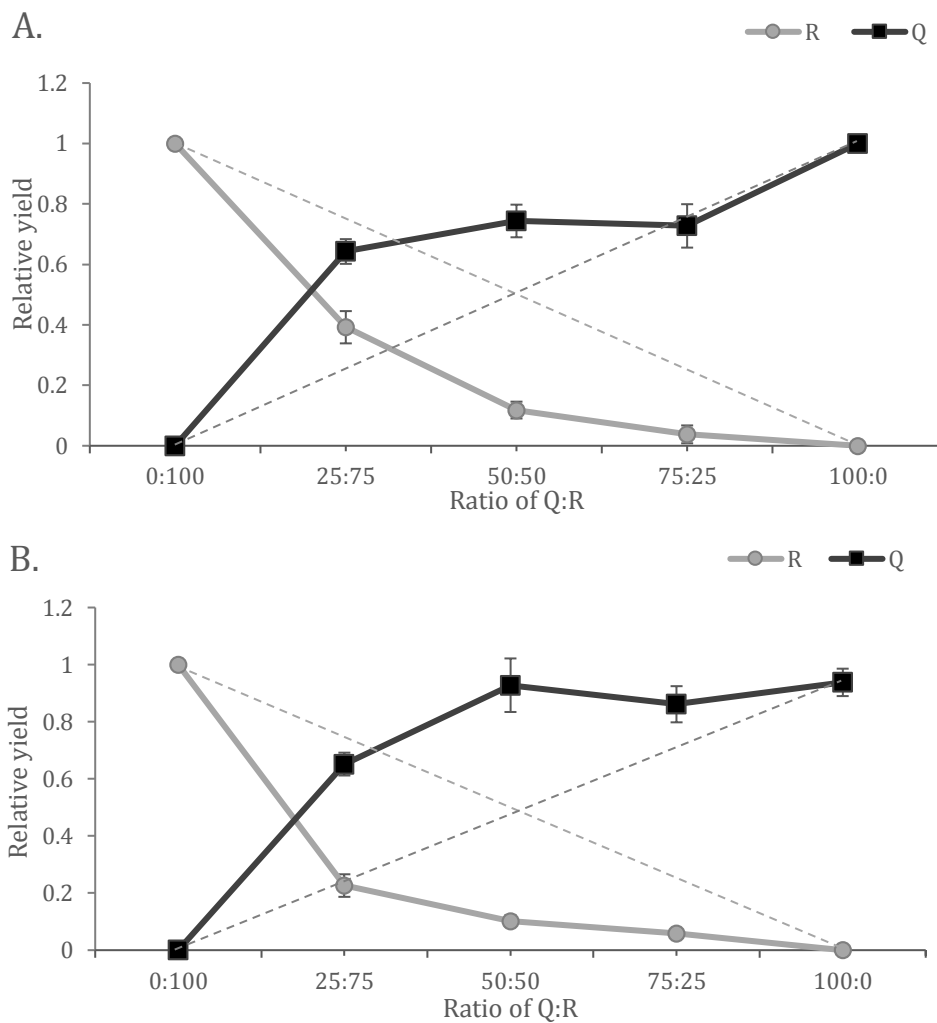
**Figure 1.**

Replacement series diagrams for relative yield of quinoa and foxtail in mixture in both low nitrogen (Run 1 panel A, Run 2 panel B) and high nitrogen (Run 1 panel C, Run 2 panel D). Error bars represent standard deviation. F=foxtail, Q=quinoa.

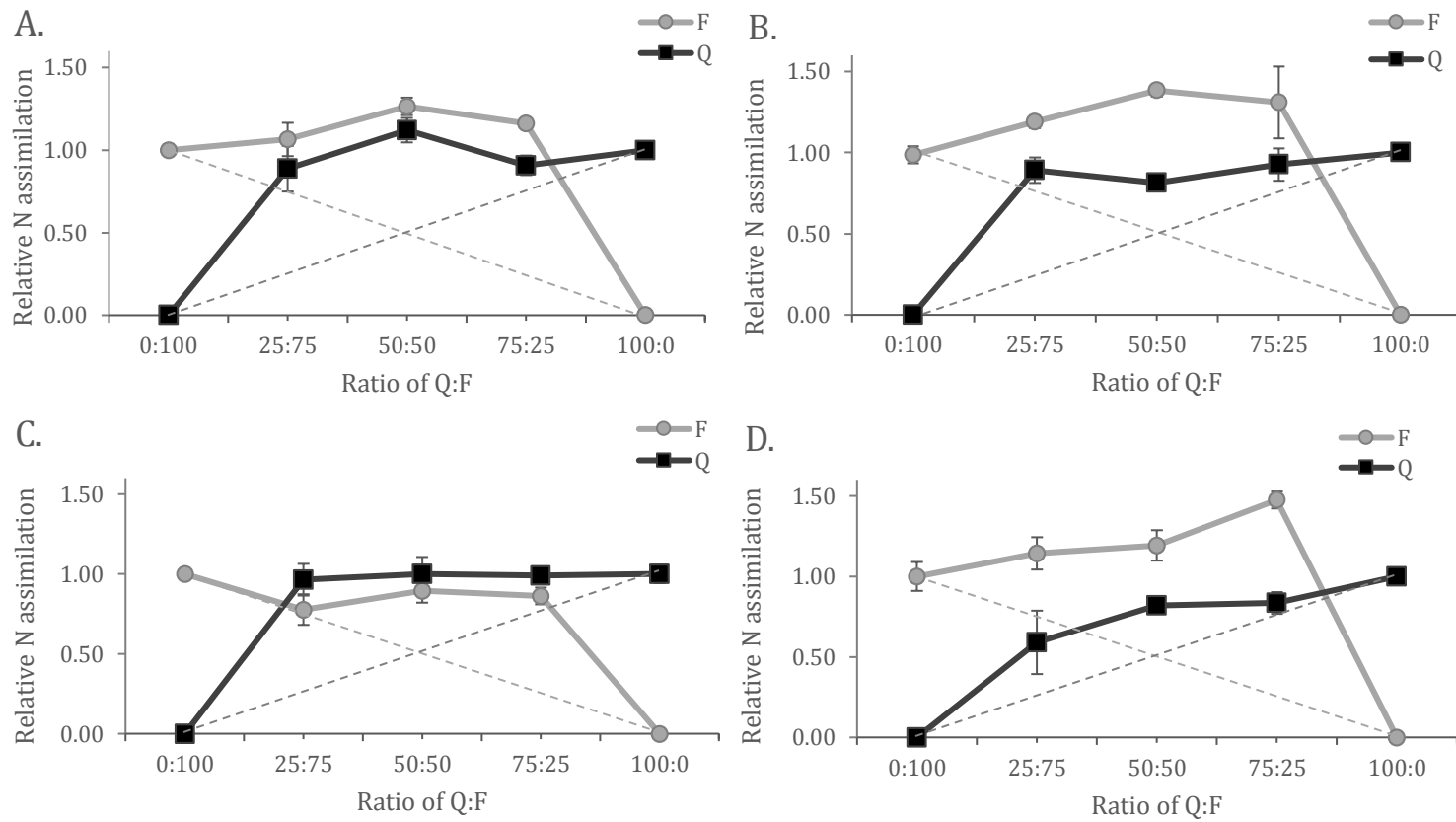


**Figure 2.**

Replacement series diagrams for relative yield of quinoa and lambsquarters in mixture in both low nitrogen (panel A) and high nitrogen (panel B). Error bars represent standard deviation. L=lambsquarters, Q=quinoa.

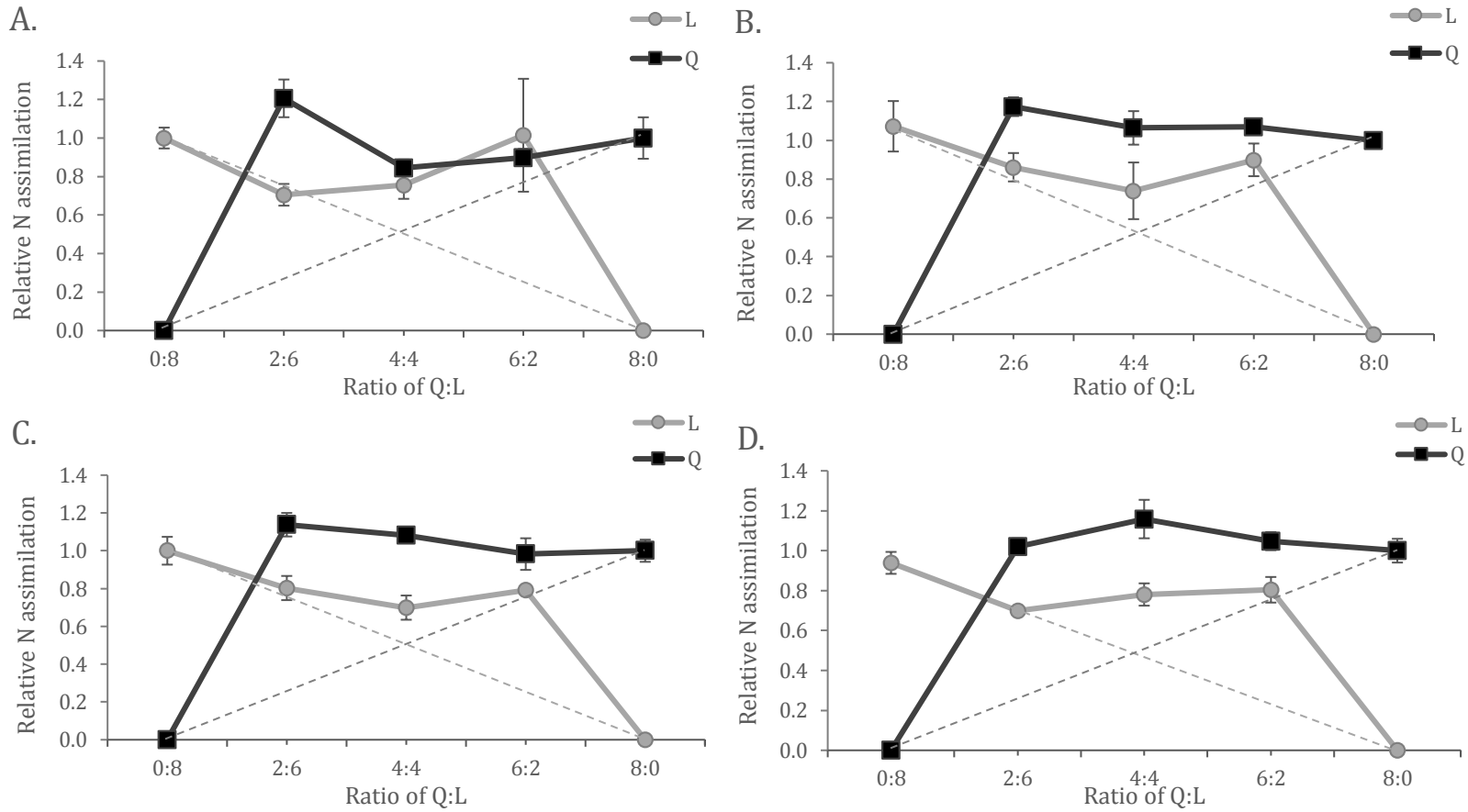


**Figure 3.** Replacement series diagrams for relative yield of quinoa and redroot in mixture in both low nitrogen (panel A) and high nitrogen (panel B). Error bars represent standard deviation. R=redroot pigweed, Q=quinoa.



**Figure 4.**

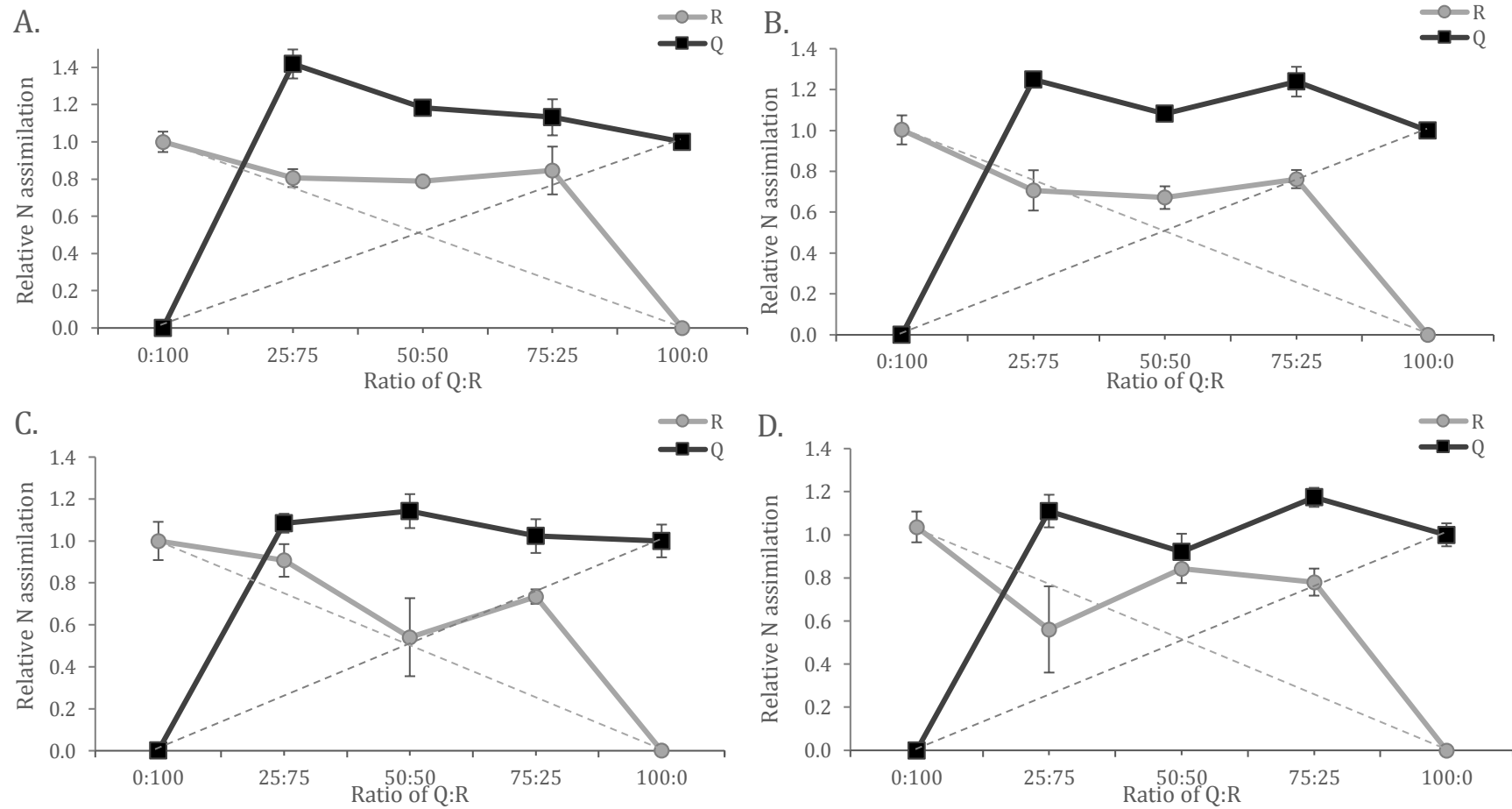
Replacement series diagrams for relative N assimilation of quinoa and foxtail in mixture in both low nitrogen (Run 1 panel A, Run 2 panel B) and high nitrogen (Run 1 panel C, Run 2 panel D). Error bars represent standard deviation. F=foxtail, Q=quinoa.



**Figure 5.**

Replacement series diagrams for relative N assimilation of quinoa and lambsquarters in mixture in both low nitrogen (Run 1 panel A, Run 2 panel B) and high nitrogen (Run 1 panel C, Run 2 panel D). Error bars represent standard deviation. L=lambsquarters, Q=quinoa.





**Figure 6.**

Replacement series diagrams for relative N assimilation quinoa and redroot in mixture in both low nitrogen (Run 1 panel A, Run 2 panel B) and high nitrogen (Run 1 panel C, Run 2 panel D). Error bars represent standard deviation. R=redroot pigweed, Q=quinoa.

CHAPTER VI: EFFECTS OF CROPPING SYSTEM HISTORY AND COMPOST  
APPLICATION ON SOIL HEALTH INDICATORS

ABSTRACT

Cropping history can have a lasting impact on soil microbial populations and their resistance and resilience to disturbance. Conditions such as weather events (drought, freeze, heat) and agrichemical inputs are common under field conditions and can be used to assess resistance and resilience. The goal of this study was to determine if cropping history and/or compost addition affect the resistance and resilience of the soil microbial community to disturbance. Soils were collected at a depth of 0-10 cm from two neighboring field sites (Millville silt loam) managed conventionally (C) primarily using mineral fertilizers with no organic matter inputs or organically (O) for a minimum of 10 years. Each soil was treated in the laboratory with a single application of composted steer manure equivalent to 11.2 Mg DM ha<sup>-1</sup> (+1) or none (+0) prior to incubation at 25 °C. Soils in each treatment were then subjected to stress: heat, freeze, drought, application of glyphosate, or no stress (control) and returned to steady state conditions after 24 hours. Microbial biomass (MB, as measured by substrate induced respiration) was assessed at 0, 1, 2, 7, 14, and 28 days after stress (DAS). Microbial biomass was higher in organic than conventional soil regardless of stress and conventionally managed soil produced more CO<sub>2</sub>-C per unit biomass, indicating reduced metabolic efficiency. Changes in MB due to stress varied, such that drought and glyphosate stress increased MB over controls while heat stress reduced it and freezing produced no change on only some sample dates.

Organically managed soils had the highest resistance to disturbance while conventionally cropped soil recovered quicker. Compost increased resilience following glyphosate and freeze stress but lowered resilience in heat stressed soil. Microbial death rate was increased in compost treated soil. The complex interactions observed in this study suggest labile carbon cycling may help predict microbial response to common agricultural stressors in future research.

## **1. Introduction**

Farming in the western United States faces steep challenges. Increased frequency and severity of heat and drought stress are forecast for the region, where agricultural soils are already intensively managed and low in organic matter. Developing cropping systems that maintain or improve soil health may be a successful approach to increasing farm sustainability (Doran, 2002). Soil health has been described as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA Natural Resources Conservation Service 2014). Indicators of soil health frequently involve measures of soil microbial biomass size and activity. Similar measurements have also been used to describe the soil response to stress (Udawatta 2010; Kumar et al. 2014; Benitez et. al, 2004). The resistance and resilience of soil microbial populations to both natural and anthropogenic stress events have been suggested as key indicators of soil health (Herrick 2000; Seybold et al. 1999; Griffiths and Philippot 2012).

The terms soil resistance and resilience stem from ecological concepts that can be applied to the soil microbial response to disturbance, such as heat or freeze events, tillage, or chemical contamination (Seybold et al., 1999; Abner and Melillo, 1991). Soil resistance describes the ability of a soil to continue to function without decline following

a disturbance (Seybold et al., 1999; Pimm 1984). Soil resilience refers to the rate and degree of recovery of a soil following disturbance (Seybold et al., 1999; Pimm 1984). Agricultural soils are constantly subjected to disturbances from both cropping practices and natural processes, which all influence microbial populations. Many factors can influence resistance and resilience, including soil type and texture as well as cropping history (Griffiths and Philippot 2012; Orwin and Wardle 2005; Royer-Tardif et al., 2010; Kumar et al. 2014; Chaer et al. 2009). Previous research on the resistance and resilience of the soil microbial biomass has focused on changes in the size, function or composition of populations in response to stress (Chaer et al., 2009; Fujino et al., 2008; Hueso et al., 2011; Kumar et al., 2014). Yet, there is little reported about the influence of labile C on microbial resistance and resilience to stress.

The amount of labile C in the soil greatly impacts microbial growth, which can be assessed by measuring soil CO<sub>2</sub> fluxes (Iqbal et al., 2010). Frequently, microbial biomass (MB) is limited by the soil concentrations of labile C and/or nitrogen (N) in soils, both of which can be supplied through agri-chemical and organic matter inputs to create favorable soil C:N ratios. However, readily available resources can have different effects on the resistance and resilience of soils (Kumar et al., 2014). Kumar et al. (2014) report the combination of chemical fertilizer and manure increased MB and resistance to heat stress, yet soils supplied only chemical fertilizers at different levels showed less tolerance to stress, suggesting a complex interaction between microbial population growth and resistance and resilience to stress events.

Management practices such as compost additions and diverse cropping systems, common on organic farms, tend to build the size and efficiency of microbial biomass

production and may increase overall soil health (Anderson and Domsch, 1990; Hueso et al., 2011). Likewise, the efficiency of the microbial biomass production has been shown to be higher in long standing systems (Anderson and Domsch, 1990). However, it is unclear if this increase in efficiency is a result of organic matter (OM) inputs or other management influences. It is also unclear if the changes in microbial population size and efficiency due to cropping system or compost inputs lead to changes in microbial resistance and resilience to stress.

In this study we compared the response of a single soil type under long-term organic or conventional management to compost amendment and or a variety of stress events. We hypothesized that soil managed organically has increased soil MB, higher metabolic efficiency, and is more resistant and resilient to stress than conventionally managed soil. Furthermore, we hypothesized the addition of compost will increase labile C, soil MB, metabolic efficiency, resistance and resilience. To test these hypotheses, organic and conventionally managed soil with and without the addition of compost was subjected to a number of stressors. Soil resistance and resilience were assessed by measuring basal respiration, readily mineralizable carbon and soil microbial biomass.

## **2. Materials and Methods**

### *2.1 Soil sampling and analyses*

Soils were sampled to a depth of 10 cm from two neighboring fields at the Greenville Experiment Station, Logan Utah. Approximately 100 random sub-samples per field were collected with 2.5 cm corer to a depth of 10 cm and then pooled. The soil type was a Millville silt loam (Table 1) under long-term cultivation in both organic and conventional management. The Greenville organic field had been managed organically

with a variety of summer and winter cover crops but without compost or other organic fertilizer additions for over ten years. The conventional field had been managed with a variable crop rotation including alfalfa, corn, and wheat with inorganic fertility inputs and herbicide applications, but no cover crops or compost additions.

Soils were sieved through a 4.0 mm screen, stored in re-sealable plastic bags and refrigerated at 4°C until processing within 7 days. Prior to treatment application (stress or compost), both soils were measured for a number of variables. Nitrate and ammonium-N ( $\text{NH}_4^+\text{-N}$ ) were extracted in 1M KCl, and analyzed by Lachat (Quickchem 8500, Hach Company, Loveland, CO) using sulfanilamide and phenate methods, respectively according to manufacturer protocols. Soil EC and pH were measured in a 1:2 soil:water suspension. Total N and total organic C were measured by combustion from air-dried soils collected in July according to the manufacturer's protocol (Skalar Primacs Total Nitrogen Analyzer, Skalar Primac SLC Carbon Analyzer, respectively, Salt Lake City, Utah).

The compost treatment was applied to each soil by mixing in a large container, adding DI water to bring the soil to 22 % gravimetric moisture content and applying compost at the rate of 11.2 Mg DM ha<sup>-1</sup> to approximately half of the soil collected from each cropping system.

Each soil was then divided into 4 replicates of the following cropping system/treatment combinations: cropping history [two levels: organic (O), and conventional (C)], compost addition [two levels: 11.2 Mg DM ha<sup>-1</sup> (+1) or none (+0)] and stress type [five levels: heat, freeze, drought, glyphosate application, and none]. Each

replicate was incubated in a re-sealable bag with a straw to allow for air exchange (Sullivan et al., 2011). Stress treatments were applied as follows in the laboratory on day zero (DAS0):

1. Heat stress at to 40 °C for 24 hours. (Kumar et al., 2014; Gregory et al., 2009);
2. Freeze stress at 0 °C for 24 hours (Kumar et al., 2014; Gregory et al., 2009);
3. Drought stress by air drying (relative humidity approximately 40%) for 24 hours (Orwin and Wardle, 2004);
4. Glyphosate addition of 0.20 g ai g<sup>-1</sup> soil to mimic field rate applications;

Prior to stress treatment, 5 g od eq soil was removed from the bags and weighed into borosilicate vials with septa. Active microbial biomass was measured using substrate induced respiration (SIR) by sequentially adding 0.5 mL of 60 g L<sup>-1</sup> aqueous solution of glucose, resting the samples for one hour uncapped, recapping the vials for two hours, and then removing headspace CO<sub>2</sub> samples for analysis (Anderson and Domsch, 1978). Readily mineralizable carbon (RMC) and soil basal respiration (BR) were also measured on the same soils at DAS 0 (prior to stress) according to Anderson and Domsch (1978). Carbon dioxide (CO<sub>2</sub>) measured in the headspace after 11 days was considered RMC. Vials were uncapped, flushed for one minute using moisture saturated air, and then recapped and the hourly rate of CO<sub>2</sub> production measured for BR after exactly two hours. An infrared gas analyzer (model 6251, LICOR Biosciences, Lincoln, NE) was used to measure CO<sub>2</sub> in the headspace. Soil dissolvable organic carbon was also measured prior to stress treatment on 10 g od wt in 50 mL water according to the manufacturers' protocol of oxidation by UV-Persulfate (Tekmar Dohrmann Phoenix 8000, Mason, OH, USA).

Following the application of stress treatments on DAS 0, replicates were incubated in a dark cupboard at 25°C until sampling. Microbial biomass response to stress, cropping system and compost was assessed using substrate induced respiration (SIR) as described above at repeated intervals, 1, 2, 7, 14, and 28 days following the application of stress events (Anderson and Domsch, 1978; Orwin and Wardle, 2004). Additionally, on DAS 28, RMC, BR and dissolved organic carbon (DOC) were also measured as described above. Bags were weighed and water added as necessary to maintain soil moisture at 22% throughout the experiment.

Microbial efficiency quotient ( $qCO_2$ ) and microbial C-loss quotient ( $qD$ ) were measured on DAS 28 using the following equations (Anderson and Domsch, 1990):

$$qCO_2 = \frac{MB}{BR}$$

$$qD = \left( \frac{MB_{t1} - MB_{t2}}{MB_{t1}} \right) / (t2 - t1)$$

## 2.2. Resistance and resilience indices

Microbial biomass was used to calculate a resistance index for soils on DAS 1 using the following equation:

$$Resistance = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

where  $C_0$  is the control value at time zero and  $D_0$  is the difference between the control and the disturbed soil at the end of the disturbance (Orwin and Wardle, 2004). This resistance index is standardized by the control soil and therefore can account for the maximum amount of change a particular stress may cause in a given soil. A resilience



index was also calculated for repeated MB measures for each stress treatment on DAS 1, 3, 7, 14, and 28 as described by Orwin and Wardle (2004) at time  $x$  ( $t_x$ ):

$$\text{Resilience at } t_x = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1$$

where  $D_x$  is the difference between the control soil and disturbed soil at time  $x$ , and  $D_0$  remains the same as above. The resilience index is standardized by the initial amount of change from a given stress and therefore captures the recovery of a given system relative to the amount of initial change from a stress.

### 2.3. Statistical analyses

Cropping system and compost treatment comprised a two-way factorial (cropping history and compost level) in a completely randomized design (CRD), where incubated soil bag was the experimental unit and day after stress (DAS) was a repeated measure. Sample date was significant in all microbial biomass measurement interactions, hence each date was analyzed separately. On Day 0 and 28, soil RMC, BR, MB, and DOC were also analyzed separately as a two-way CRD. A mean was computed at the treatment level for all subsamples. The response variables of RMC, BR, MB, DOC and resistance and resilience indices were assessed using analyses of variance with PROC GLIMMIX in the Statistical Analysis System for Windows version 9.4 (SAS Institute, Cary, NC). Variables were square-root or log transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. Multiple means comparisons were adjusted using Tukey's method to control for family-wise Type I error rate. Pairwise comparisons between means were aided by the macro PDMIX800 (Saxton, 1998).

### 3. Results

Cropping history had a large effect on soil microbial and C measures before stress was applied. Baseline total organic C and N were almost double in the organically managed fields (Table 2). At the beginning of the trial (Table 3), organically managed soil had greater RMC, BR and MB than the conventional field, regardless of compost level ( $p < 0.0001$  for all). At the same time, conventional soil treated with compost had more RMC than without compost ( $p < 0.0001$ ). This pattern was also observed in the MB on DAS 1, 7 and 28 (Table 4). These results suggest the history of organic management increased soil carbon stores, which were then unresponsive to additional OM inputs whereas microbial measures in the conventional system were significantly impacted by a single, moderate rate of compost. However, there were notable exceptions to these trends in MB over time. On DAS 14, the compost effect was reversed. At this sample time, within each cropping system and stress treatment, soil without compost had higher MB than amended soil. Organically managed soil had greater MB than conventionally managed soil (Table 5). This may be due to variability or suggests readily available C and N sources within compost treatments had been utilized.

The impact of stress treatment on MB also changed with sample date and imposed stress condition. Regardless of applied stress, organically managed soil had higher MB than conventionally managed soil on DAS 2, 14 and 28. When subjected to drying and re-wetting, the MB of the drought stressed soil increased over the control on DAS 1, 7 and 14 (Table 4 and 5). A short-lived increase in MB over the control was also observed on glyphosate treated soils on DAS 1, with no other differences observed (Table 4). The application of heat stress reduced MB over all other stressors on DAS 1 and DAS 7 with

the exception of freeze stress on DAS 7, which did not differ from the heat stressed soil (Table 4). On DAS 2, a complicated interaction between stress, cropping systems, and compost was observed indicating the addition of compost to organically managed soil increased MB in all stress treatments except heat, while no differences were noted in any conventionally managed/compost treatments. Conversely on DAS 14, heat stressed soil had more MB than the control in both organic and conventional systems. Freezing and thawing soils did not change the MB from control soils on any sample day.

Following the end of the trial, both cropping system and compost impacted microbial measures (Table 7). Readily mineralizable C, BR, MB and DOC were all higher in organically managed than conventional soil ( $p < 0.0001$ ) and RMC was higher with the addition of compost only in conventionally managed soil ( $p < 0.0001$ ). Conventional soil also had higher  $\text{CO}_2\text{-C}$  produced per unit MB ( $q\text{CO}_2$ ) than organically managed soil ( $p < 0.0001$ ). Microbial biomass response to compost was mixed. Organically managed soil without compost had greater MB than with compost ( $p = 0.0085$ ); on the other hand, compost treated conventional soil had higher MB than without (Table 7). The microbial death rate ( $q\text{D}$ ) also increased with compost addition (Table 9).

Limited impact of stress treatments were observed at the end of the study. Drought stress reduced minC more than any other stress treatment, regardless of compost level (Table 7). Mineralizable C and BR were higher in soil subjected to heat stress with compost than any other stress treatment with compost. These MB responses to stress are mirrored in the  $q\text{CO}_2$  results. Heat stress increased  $q\text{CO}_2$  over all other stress treatments and drought stress had the lowest  $q\text{CO}_2$  with all other treatments in between (Table 8).

Heat stress also increased MB death rate in conventional, but not organic soil (Table 9). While there was no difference in DOC at DAS 0, on DAS 28, both heat and glyphosate stress had higher DOC than the control soils (Table 7).

Cropping system and compost played a role in both the resilience and resistance of MB to disturbances. Resistance was highest in soils with an organic cropping history while resilience was generally higher in the conventional cropping system than the organic system. Averaged over all compost levels and imposed stress, organic soil was more resistant ( $p=0.017$ ) to disturbance than conventional soil (Figure 1). No other factors were significant in predicting the resistance index of soil. The resilience to stress was also affected by cropping system over time. In drought stressed soils, conventional soil had higher resilience than organic ( $p=0.001$ ) (Table 6). A significant day\*cropping system interaction in heat-stressed soil revealed conventionally managed soil had greater ( $p=0.003$ ) resilience than organic on DAS 7 only (Table 6 and Figure 3). Similarly, in glyphosate treated soil, conventionally managed soil had a greater ( $p=0.05$ ) resilience index than organically managed soil on DAS2 with no other differences observed (Figure 2).

Compost effects were most pronounced in the resilience index in heat, freeze and glyphosate stress treatments. The addition of compost to organically managed soil seemed to decrease the ability of the soil to recover from heat stress. Averaged over all sample dates, heat stressed-conventional soil had higher resilience than organically managed soil with compost ( $p=0.003$  and  $p=0.009$ ), while organically-managed soil without compost was intermediate (Table 6). In the freeze-stressed soil, the opposite

effect was observed in the conventional system where compost amended-conventionally cropped soil had greater resilience, averaged over all dates, than any other cropping system/compost combination (greater than C+0 at  $p=0.005$ , O+0  $p=0.011$ , and O1  $p<0.0001$ ) (Table 6). Compared to un-amended soil, the benefit of compost was greater in the glyphosate-stressed soil where the addition of compost increased the resilience index ( $p=0.017$ ). Compost was not a significant factor in the resilience to drought stress in this study (Table 6).

#### **4. Discussion**

Organically managed soil in this study had a larger MB, more organic C stores, utilized C more efficiently, and showed higher resistance to disturbance from stress than conventional soil. Soils under diverse crop rotations have been shown to have lower  $qCO_2$  than monoculture alone (Anderson and Domsch, 1990). In contrast to our hypothesis, microbial biomass recovery of organically managed soil was slower than the conventional system. Kumar et al. (2014) also observed long-term OM inputs increased the stability, or resistance compared to a system with only chemical fertilizer. However, unlike our findings, they also report the system receiving OM recovered more quickly, possibly due to the combination of OM inputs and a complete chemical fertilizer resulting in highest levels of total N and soluble C and N.

Although not monitored throughout the course of this study, soil nutrient availability likely played a role in microbial recovery from stress. The total N and total organic C in the conventional system was half of that in the organic system but the available N at the beginning of the trial was seven times greater. The application of stress

may have preferentially affected portions of the microbial population responsible for N-mineralization processes and therefore had a longer lasting effect on low N availability in organically managed soils (Hueso et al., 2011). A short lived increase in MB in drought and glyphosate soils over the control soil likely indicates a response to nutrient pulses from different sources. In the drought soils, microbes killed during stress events can serve as readily available resources for the tolerant portion of MB (Hueso et al., 2011), while the addition of glyphosate itself provides an external input with a low C:N ratio (Lancaster et al. 2010; Haney et al., 2002).

The impact of compost was less clear than cropping system effects. Compost addition increased labile soil organic matter (SOM) as measured in this study by RMC and DOC, increased the microbial death rate, and increased MB in conventionally cropped soils. The lack of compost effects on microbial biomass in the organically managed system indicates a system that is above a threshold of OM stores, where the benefits of additional C inputs are not observed in a short time-frame study. The addition of compost also had little impact on the response of soils to stress treatments because resilience was only increased in glyphosate treated soils and conventionally managed soils subjected to freeze stress. Hueso et al., (2011) describe a similar response in an incubation study using arid soil, where a single compost addition increased water retention under drought conditions and increased measures of microbial activity and size; however, recovery between amended and non-amended soils was similar. The authors suggest this is due to insufficient time under stress to effectively alter the community structure in which species are well adapted to imposed stress (Hueso et al., 2011).

Maintaining or increasing SOM has been suggested as a key strategy to improve the sustainability of farms and sequester soil C (Robertson and Grace, 2004). More recently, research has focused on identifying changes in turnover rate of SOM due to common agrichemical inputs with favorable C:N. Glyphosate- and heat-treated soils had higher DOC in the conventional soils without compost at the end of the trial indicating increased solubilization of carbon sources. While the mineralization of organic matter provides plant-available nutrients, an enhanced mineralization process may produce unwanted effects such as accelerated depletion of SOM and/or loss of available nutrients from the root zone. If SOM turnover rates are increased by the addition of readily available substrates, more recalcitrant OM may then be subject to breakdown (Guenet et al., 2012; Hamer and Marschner, 2005a, 2005b).

The resilience of agricultural soils to stress is likely a complex interaction among management strategies such as plant composition, organic amendments, and tillage events (Chaer et al. 2009; Orwin and Wardle 2005; Royer-Tardif et al., 2010). Orwin and Wardle (2005) conclude that while community structure has an influence on resistance and resilience, many other factors influence response to stress, such as nutrient availability and the timing of sampling. Royer-Tardif et al. (2010) observed that mixed stands of trees had a more robust community structure than monoculture and were more resistant to disturbance, while resilience in these systems was linked to soil type which the authors attribute to bacterial/fungal ratios and nutrient availability. The ratio of bacteria to fungi may be an important measure in the soils of this study and could be a contributing factor to the slow recovery of the organic system to disturbances (de Vries et al., 2012). In examining the microbial functional response to stress, Chaer et al. (2009)

conclude a more effective measure of microbial stability in response to cultivation strategies can be seen through a host of specific functional tests such as enzyme assays.

Orwin and Wardle (2005) also examined the effects of plant composition on microbial communities and reported individual plant species, but not the diversity of species, affected both resistance and resilience measures due to suspected differences in nutrient resources. The cropping systems in our study were managed under very different plant compositions prior to our sampling; the conventional system had been maintained as a bare fallow, while the organic system was under diverse plant cover including quinoa, clover, hairy vetch and wheat. The specific interaction of these crops with microbial populations is not known. While not measured in this study, a more robust community structure in the organic system may be the cause of increased resistance. Evaluation of microbial species richness combined with functional enzyme assays would be valuable to determine contributing factors to resistance and resilience measures in this study.

## **5. Conclusion**

Organically managed soils had higher MB, more efficient MB (respiration per unit biomass), were more resistant to imposed environmental stress, and showed little impact of compost addition. Conventionally managed soils had a higher resilience index at two sample points following heat stress or glyphosate application. The addition of compost also increased MB in conventionally managed soils. The response of soils to stress treatments likely was affected by the structure and degree of diversity in microbial populations as well as the available nutrients. Future work focusing on changes in



community composition and the mineralization of organic matter following stress events could provide useful insights into driving factors of resistance and resilience.

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## Tables and figures

**Table 1**

Soil characteristics for both conventional and organic plots at the Utah State University Greenville Research station, Logan, UT.

|   | Conventional        | Organic |
|---|---------------------|---------|
| Classification                                      | Millville silt loam |         |
| pH (2:1)  | 8.5                 | 8.5     |
| EC (2:1) ( $\mu\text{S}/\text{cm}$ )                | 112                 | 161     |
| Total nitrogen (%)                                  | 0.21                | 0.41    |
| Total organic carbon (%)                            | 0.62                | 1.12    |
| Nitrate <sup>1</sup> ( $\mu\text{g g soil}^{-1}$ )  | 9.61                | 1.26    |
| Ammonium <sup>1</sup> ( $\mu\text{g g soil}^{-1}$ ) | 0.55                | 0.18    |

<sup>1</sup> Soil extractable nitrate and ammonium as a snapshot of plant available soil N at time of sampling in October 2014.

**Table 2**  
Composted steer manure nutrient analysis (analysis on air-dried compost).

| Parameters      | Value |
|-----------------|-------|
| Moisture (%)    | 4.9   |
| pH (2:1)        | 8     |
| EC (2:1) (dS/m) | 4.32  |
| N (%)           | 1.54  |
| C (%)           | 24.5  |
| P (%)           | 0.60  |
| K (%)           | 1.32  |
| Ca (%)          | 3.72  |
| Mg (%)          | 0.74  |
| S (%)           | 0.33  |
| Na (mg/kg)      | 3410  |
| B (mg/kg)       | 17.3  |
| Zn (mg/kg)      | 211   |
| Cu (mg/kg)      | 31.2  |
| Fe (mg/kg)      | 5980  |
| Mn (mg/kg)      | 253   |

**Table 3**

Mean values for readily mineralizable carbon (RMC), basal respiration (BR), microbial biomass (MB) and dissolved organic carbon (DOC) at Day 0 prior to application of environmental stress. Letters indicate significant differences ( $p < 0.05$ ) within date of sampling.

|                 |                | Day 0                              |   |                             |                                |
|-----------------|----------------|------------------------------------|---|-----------------------------|--------------------------------|
|                 |                | Readily<br>Mineralizable<br>Carbon | Soil<br>Respiration                               | Microbial<br>Biomass        | Dissolved<br>Organic<br>Carbon |
|                 |                | (mg kg soil <sup>-1</sup> )        | (mg kg soil <sup>-1</sup><br>hour <sup>-1</sup> ) | (mg kg soil <sup>-1</sup> ) | (ppm)                          |
| Crop system     |                |                                    |   |                             |                                |
|                 | C              | 15.3 <sup>B</sup>                  | 3.87 <sup>B</sup>                                 | 254 <sup>B</sup>            | 0.31                           |
|                 | O              | 44.2 <sup>A</sup>                  | 7.28 <sup>A</sup>                                 | 434 <sup>A</sup>            | 0.33                           |
| Compost         |                |                                    |   |                             |                                |
|                 | +0             | 27.3 <sup>B</sup>                  | 5.27  | 338                         | 0.29                           |
|                 | +1             | 32.2 <sup>A</sup>                  | 5.87  | 350                         | 0.35                           |
| System*compost  |                |                                    |   |                             |                                |
|                 | C+0            | 12.2 <sup>C</sup>                  | 3.72  | 248.                        | 0.29                           |
|                 | C+1            | 18.3 <sup>B</sup>                  | 4.01  | 259                         | 0.33                           |
|                 | O+0            | 42.3 <sup>A</sup>                  | 6.83  | 428                         | 0.29                           |
|                 | O+1            | 46.1 <sup>A</sup>                  | 7.74  | 440                         | 0.36                           |
| <b>p-values</b> |                |                                    |   |                             |                                |
|                 | Crop system    | <0.001                             | <0.001  | <0.001                      | 0.730                          |
|                 | Compost        | <0.001                             | 0.191   | 0.131                       | 0.286                          |
|                 | System*compost | 0.002                              | 0.6545  | 0.953                       | 0.823                          |

**Table 4**

Mean values for microbial biomass on days after stress (DAS) 1 and 7. Letters indicate significant differences ( $p < 0.05$ ) within date of sampling.

|                            |           | Microbial Biomass<br>(mg kg soil <sup>-1</sup> ) |                   |
|----------------------------|-----------|--|-------------------|
|                            |           | DAS 1  | DAS 7             |
| Stress                     | Control   | 307 <sup>B</sup>                                 | 322 <sup>B</sup>  |
|                            | Drought   | 345 <sup>A</sup>                                 | 349 <sup>A</sup>  |
|                            | Freeze    | 326 <sup>AB</sup>                                | 314 <sup>BC</sup> |
|                            | Heat      | 277 <sup>C</sup>                                 | 292 <sup>C</sup>  |
|                            | Herbicide | 329 <sup>A</sup>                                 | 321 <sup>AB</sup> |
| Crop system*compost        | C+0       | 204 <sup>C</sup>                                 | 198 <sup>C</sup>  |
|                            | C+1       | 231 <sup>B</sup>                                 | 210 <sup>B</sup>  |
|                            | O+0       | 409 <sup>A</sup>                                 | 442 <sup>A</sup>  |
|                            | O+1       | 423 <sup>A</sup>                                 | 429 <sup>A</sup>  |
| <b>p-values</b>            |           |  |                   |
| Crop system                |           | <0.001   | <0.001            |
| Compost                    |           | <0.001   | 0.192             |
| Stress                     |           | <0.001   | <0.001            |
| Stress*crop system         |           | 0.212  | 0.541             |
| Crop system*compost        |           | 0.010  | 0.002             |
| Stress*compost             |           | 0.730  | 0.541             |
| Crop system*compost*stress |           | 0.739  | 0.472             |



**Table 5**

Mean values for microbial biomass on days after stress (DAS) 2, 14 and 28. Letters indicate significant differences ( $p < 0.05$ ) within date of sampling.

|   |              | Microbial Biomass<br>(mg kg soil <sup>-1</sup> ) |                   |                   |                  |
|---|--------------|--|-------------------|-------------------|------------------|
|   |              | DAS 2  | DAS 14            |                   | DAS 28           |
| Stress or stress*compost                          |              |  | <u>+0</u>         | <u>+1</u>         |                  |
|   | Control      | 359  | 287 <sup>BC</sup> | 245 <sup>D</sup>  | 194 <sup>B</sup> |
|   | Drought      | 362  | 314 <sup>A</sup>  | 281 <sup>BC</sup> | 210 <sup>A</sup> |
|   | Freeze       | 343  | 295 <sup>AB</sup> | 259 <sup>CD</sup> | 196 <sup>B</sup> |
|   | Heat         | 338  | 314 <sup>AB</sup> | 313 <sup>A</sup>  | 189 <sup>B</sup> |
|   | Glyphosate   | 375  | 300 <sup>AB</sup> | 247 <sup>D</sup>  | 196 <sup>B</sup> |
| Crop system*compost                               |              |  |                   |                   |                  |
| C   | Without (+0) | 246 <sup>C</sup>                                 | 202 <sup>C</sup>  |                   | 131 <sup>C</sup> |
|   | With (+1)    | 258 <sup>C</sup>                                 | 191 <sup>D</sup>  |                   | 137 <sup>B</sup> |
| O   | Without (+0) | 402 <sup>B</sup>                                 | 396 <sup>A</sup>  |                   | 259 <sup>A</sup> |
|   | With (+1)    | 516 <sup>A</sup>                                 | 346 <sup>B</sup>  |                   | 260 <sup>A</sup> |
| Stress*crop system or crop system*compost *stress |              |  | <u>+0</u>         | <u>+1</u>         |                  |
| C   | Control      | 254 <sup>D</sup>                                 | 266 <sup>D</sup>  | 184 <sup>E</sup>  | 130 <sup>C</sup> |
|   | Drought      | 240 <sup>D</sup>                                 | 240 <sup>D</sup>  | 200 <sup>D</sup>  | 136 <sup>C</sup> |
|   | Freeze       | 231 <sup>D</sup>                                 | 277 <sup>D</sup>  | 187 <sup>DE</sup> | 133 <sup>C</sup> |
|   | Heat         | 259 <sup>D</sup>                                 | 241 <sup>D</sup>  | 218 <sup>C</sup>  | 136 <sup>C</sup> |
|   | Glyphosate   | 245 <sup>D</sup>                                 | 265 <sup>D</sup>  | 193 <sup>DE</sup> | 136 <sup>C</sup> |
| O   | Control      | 374 <sup>C</sup>                                 | 542 <sup>A</sup>  | 348 <sup>B</sup>  | 257 <sup>B</sup> |
|   | Drought      | 404 <sup>BC</sup>                                | 563 <sup>A</sup>  | 395 <sup>A</sup>  | 284 <sup>A</sup> |
|   | Freeze       | 376 <sup>C</sup>                                 | 489 <sup>AB</sup> | 367 <sup>AB</sup> | 258 <sup>B</sup> |
|   | Heat         | 433 <sup>BC</sup>                                | 419 <sup>BC</sup> | 392 <sup>A</sup>  | 242 <sup>B</sup> |
|   | Glyphosate   | 422 <sup>BC</sup>                                | 568 <sup>A</sup>  | 353 <sup>B</sup>  | 256 <sup>B</sup> |
| <b>p-values</b>                                   |              |  |                   |                   |                  |
| Crop system                                       |              | <0.001   | <0.001            |                   | <0.001           |
| Compost   |              | <0.001   | <0.001            |                   | 0.011            |
| Stress  |              | <0.001   | <0.001            |                   | <0.001           |
| Stress*compost                                    |              | <0.001   | <0.001            |                   | 0.562            |
| Crop system*compost                               |              | <0.001   | 0.001             |                   | 0.047            |
| Stress*crop system                                |              | 0.012  | 0.047             |                   | <0.001           |
| Crop system*compost*stress                        |              | 0.033  | 0.059             |                   | 0.391            |

**Table 6**Mean microbial resilience by stress. Letters indicate significant differences at  $p < 0.05$ .

|   |     | Stress              |                     |                     |                     |                     |                     |
|---|-----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|   |     | Heat                | Freeze              | Drought             | Glyphosate          |                     |                     |
| Crop system                                   |     |                     |                     |                     |                     |                     |                     |
|   | C   | 0.247 <sup>A</sup>  | 0.377 <sup>A</sup>  | 0.375 <sup>A</sup>  | 0.336               |                     |                     |
|   | O   | -0.005 <sup>B</sup> | 0.148 <sup>B</sup>  | 0.064 <sup>B</sup>  | 0.242               |                     |                     |
| Compost                                       |     |                     |                     |                     |                     |                     |                     |
|   | +0  | 0.181 <sup>A</sup>  | 0.230               | 0.203               | 0.201 <sup>B</sup>  |                     |                     |
|   | +1  | 0.061 <sup>B</sup>  | 0.295               | 0.236               | 0.377 <sup>A</sup>  |                     |                     |
| Days after stress (DAS)<br>or DAS*crop system |     |                     |                     |                     |                     |                     |                     |
|   | 1   | $\frac{C}{0^B}$     | $\frac{O}{0^B}$     | 0 <sup>B</sup>      | 0 <sup>B</sup>      | $\frac{C}{0^{BC}}$  | $\frac{O}{0^{BC}}$  |
|   | 2   | -0.124 <sup>B</sup> | -0.280 <sup>B</sup> | -0.241 <sup>B</sup> | 0.122 <sup>AB</sup> | 0.127 <sup>B</sup>  | -0.401 <sup>C</sup> |
|   | 7   | 0.644 <sup>A</sup>  | -0.116 <sup>B</sup> | 0.401 <sup>A</sup>  | 0.453 <sup>A</sup>  | 0.514 <sup>AB</sup> | 0.381 <sup>AB</sup> |
|   | 14  | 0.062 <sup>B</sup>  | -0.042 <sup>B</sup> | 0.497 <sup>A</sup>  | 0.099 <sup>AB</sup> | 0.365 <sup>AB</sup> | 0.618 <sup>A</sup>  |
|   | 28  | 0.653 <sup>A</sup>  | 0.413 <sup>A</sup>  | 0.655 <sup>A</sup>  | 0.424 <sup>A</sup>  | 0.672 <sup>A</sup>  | 0.611 <sup>A</sup>  |
| Crop system*compost                           |     |                     |                     |                     |                     |                     |                     |
|   | C+0 | 0.222 <sup>A</sup>  | 0.218 <sup>B</sup>  | 0.350               | 0.244               |                     |                     |
|   | C+1 | 0.271 <sup>A</sup>  | 0.536 <sup>A</sup>  | 0.401               | 0.427               |                     |                     |
|   | O+0 | 0.139 <sup>AB</sup> | 0.243 <sup>B</sup>  | 0.057               | 0.157               |                     |                     |
|   | O+1 | -0.148 <sup>B</sup> | 0.053 <sup>B</sup>  | 0.071               | 0.327               |                     |                     |
| <b>p-values</b>                               |     |                     |                     |                     |                     |                     |                     |
| Crop system                                   |     | 0.003               | 0.001               | 0.001               | 0.193               |                     |                     |
| Compost                                       |     | 0.138               | 0.317               | 0.713               | 0.017               |                     |                     |
| DAS   |     | <0.001              | <0.001              | 0.007               | <0.001              |                     |                     |
| Crop system*compost                           |     | 0.039               | 0.002               | 0.835               | 0.931               |                     |                     |
| Crop system*DAS                               |     | 0.035               | 0.235               | 0.247               | 0.022               |                     |                     |
| Compost*DAS                                   |     | 0.351               | 0.075               | 0.790               | 0.403               |                     |                     |
| Crop system*compost*DAS                       |     | 0.294               | 0.023               | 0.388               | 0.067               |                     |                     |

**Table 7**

Mean values of readily mineralizable carbon (RMC), basal respiration (BR), microbial biomass (MB) and dissolved organic carbon (DOC) on Day 28. Letters indicate significant differences ( $p < 0.05$ ) within date of sampling.

|                            |            |  | Day After Stress 28   |  |   |  |
|----------------------------|------------|--|---|--|---|--|
|                            |            |  | Readily<br>Mineralizable<br>Carbon<br>(mg kg soil <sup>-1</sup> ) | Soil<br>Respiration<br>(mg kg soil <sup>-1</sup><br>hour <sup>-1</sup> ) | Microbial<br>Biomass<br>(mg kg soil <sup>-1</sup> ) | Dissolved<br>Organic Carbon<br>(ppm)     |
| <b>Crop system*compost</b> |            |  |   |  |   |  |
| C                          | +0         |  | 4.57 <sup>C</sup>   | 1.58 <sup>B</sup>  | 138 <sup>D</sup>                                    |  |
|                            | +1         |  | 5.25 <sup>B</sup>   | 1.63 <sup>B</sup>  | 142 <sup>C</sup>                                    |  |
| O                          | +0         |  | 10.2 <sup>A</sup>   | 2.10 <sup>A</sup>  | 251 <sup>A</sup>                                    |  |
|                            | +1         |  | 9.69 <sup>A</sup>   | 2.07 <sup>A</sup>  | 242 <sup>B</sup>                                    |  |
| <b>Stress*crop system</b>  |            |  |   |  |   |  |
| C                          | Control    |  | 4.95 <sup>D</sup>   | 1.58 <sup>E</sup>  | 138 <sup>D</sup>                                    |  |
|                            | Drought    |  | 3.81 <sup>E</sup>   | 1.48 <sup>E</sup>  | 141 <sup>CD</sup>                                   |  |
|                            | Freeze     |  | 5.00 <sup>D</sup>   | 1.56 <sup>E</sup>  | 138 <sup>D</sup>                                    |  |
|                            | Heat       |  | 5.56 <sup>D</sup>   | 1.83 <sup>D</sup>  | 147 <sup>C</sup>                                    |  |
| O                          | Glyphosate |  | 5.25 <sup>D</sup>   | 1.57 <sup>E</sup>  | 137 <sup>D</sup>                                    |  |
|                            | Control    |  | 10.3 <sup>AB</sup>  | 2.10 <sup>ABC</sup>  | 249 <sup>AB</sup>                                   |  |
|                            | Drought    |  | 8.33 <sup>C</sup>   | 1.95 <sup>CD</sup>   | 258 <sup>A</sup>                                    |  |
|                            | Freeze     |  | 10.4 <sup>AB</sup>  | 2.11 <sup>AB</sup>   | 246 <sup>B</sup>                                    |  |
|                            | Heat       |  | 11.2 <sup>A</sup>   | 2.23 <sup>A</sup>  | 239 <sup>B</sup>                                    |  |
|                            | Glyphosate |  | 9.70 <sup>B</sup>   | 2.04 <sup>BC</sup>   | 242 <sup>B</sup>                                    |  |
| <b>Stress*compost</b>      |            |  |   |  |   |  |
| +0                         | Control    |  | 7.93 <sup>B</sup>   | 1.87 <sup>BC</sup>   | 197 <sup>ABC</sup>                                  | 1.46 <sup>FG</sup> 3.06 <sup>AB</sup>    |
|                            | Drought    |  | 5.88 <sup>C</sup>   | 1.67 <sup>D</sup>  | 203 <sup>A</sup>                                    | 1.88 <sup>DEF</sup> 3.06 <sup>AB</sup>   |
|                            | Freeze     |  | 7.63 <sup>B</sup>   | 1.81 <sup>C</sup>  | 192 <sup>BC</sup>                                   | 1.11 <sup>G</sup> 3.64 <sup>AB</sup>     |
|                            | Heat       |  | 7.69 <sup>B</sup>   | 1.99 <sup>AB</sup>   | 191 <sup>BC</sup>                                   | 1.80 <sup>DEF</sup> 4.15 <sup>A</sup>    |
| +1                         | Glyphosate |  | 7.90 <sup>B</sup>   | 1.85 <sup>BC</sup>   | 192 <sup>BC</sup>                                   | 3.08 <sup>AB</sup> 3.92 <sup>AB</sup>    |
|                            | Control    |  | 7.30 <sup>B</sup>   | 1.80 <sup>CD</sup>   | 191 <sup>BC</sup>                                   | 1.65 <sup>EFG</sup> 2.47 <sup>BCDE</sup> |
|                            | Drought    |  | 6.26 <sup>C</sup>   | 1.76 <sup>CD</sup>   | 197 <sup>ABC</sup>                                  | 1.93 <sup>CDEF</sup> 3.16 <sup>AB</sup>  |
|                            | Freeze     |  | 7.74 <sup>B</sup>   | 1.87 <sup>BC</sup>   | 193 <sup>ABC</sup>                                  | 1.46 <sup>FG</sup> 2.96 <sup>ABC</sup>   |
|                            | Heat       |  | 9.03 <sup>A</sup>   | 2.08 <sup>A</sup>  | 196 <sup>AB</sup>                                   | 2.79 <sup>ABCD</sup> 3.17 <sup>AB</sup>  |
|                            | Glyphosate |  | 7.05 <sup>B</sup>   | 1.76 <sup>CD</sup>   | 187 <sup>C</sup>                                    | 2.74 <sup>ABCD</sup> 3.64 <sup>AB</sup>  |
| <b>p-values</b>            |            |  |   |  |   |  |
| Crop system                |            |  | <0.001  | <0.001   | <0.001  | <0.001                                   |
| Compost                    |            |  | 0.018   | 0.412  | 0.602   | 0.962                                    |
| Stress                     |            |  | <0.001  | <0.001   | 0.002   | <0.001                                   |
| Crop system*compost        |            |  | <0.001  | 0.046  | <0.001  | 0.001                                    |
| Stress*crop system         |            |  | 0.026   | 0.046  | <0.001  | <0.001                                   |
| Stress*compost             |            |  | <0.001  | 0.009  | 0.006   | 0.719                                    |
| Stress*compost*crop system |            |  | 0.260   | 0.570  | 0.628   | 0.012                                    |

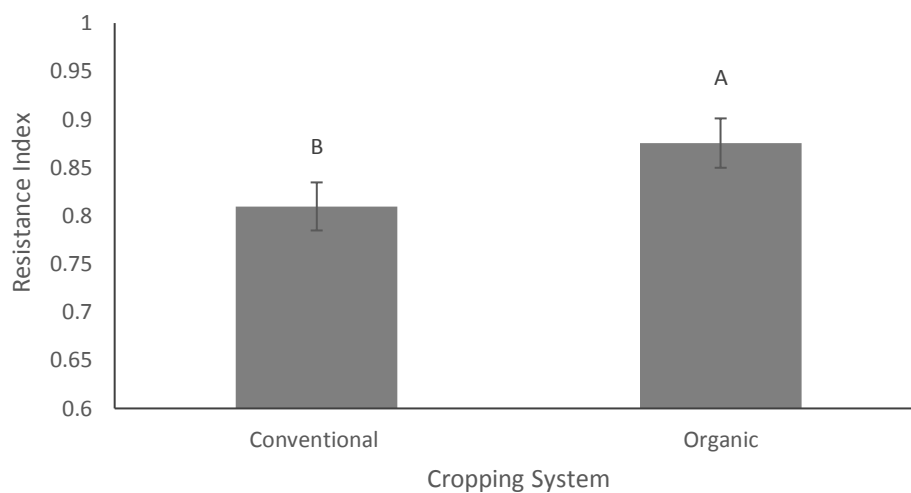
**Table 8**  
Mean  $qCO_2$ . Letters indicate significant differences at  $p < 0.05$ .

|                 |            | $qCO_2$              |
|-----------------|------------|----------------------|
| Crop system     |            |                      |
|                 | C          | 0.011 <sup>A</sup>   |
|                 | O          | 0.008 <sup>B</sup>   |
| Stress          |            |                      |
|                 | Control    | 0.010 <sup>B</sup>   |
|                 | Drought    | 0.009 <sup>C</sup>   |
|                 | Freeze     | 0.010 <sup>B</sup>   |
|                 | Heat       | 0.011 <sup>A</sup>   |
|                 | Glyphosate | 0.010 <sup>B</sup>   |
| Compost*stress  |            |                      |
| +0              | Control    | 0.010 <sup>BC</sup>  |
|                 | Drought    | 0.009 <sup>D</sup>   |
|                 | Freeze     | 0.010 <sup>BC</sup>  |
|                 | Heat       | 0.011 <sup>AB</sup>  |
|                 | Glyphosate | 0.010 <sup>ABC</sup> |
| +1              | Control    | 0.010 <sup>C</sup>   |
|                 | Drought    | 0.009 <sup>CD</sup>  |
|                 | Freeze     | 0.010 <sup>ABC</sup> |
|                 | Heat       | 0.011 <sup>A</sup>   |
|                 | Glyphosate | 0.010 <sup>C</sup>   |
| <b>p-values</b> |            |                      |
| Crop system     |            | <0.001               |
| Stress          |            | <0.001               |
| Compost*stress  |            | 0.045                |

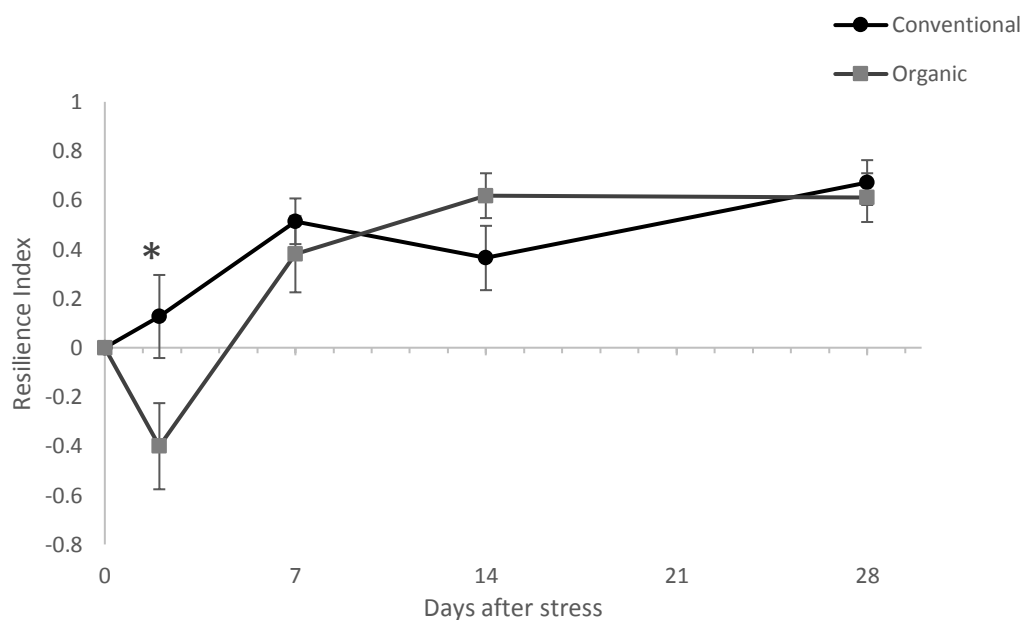
**Table 9**

Mean microbial death rate per day (qD) from days 1 to 28. Letters indicate significant differences at  $p < 0.05$ .

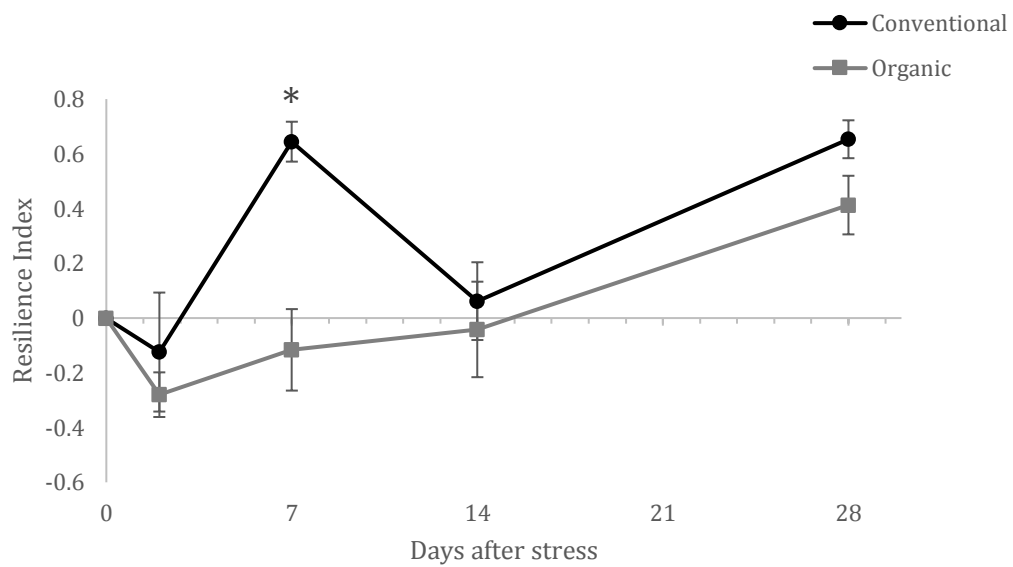
|                        |            | qD x 10 <sup>-2</sup> |
|------------------------|------------|-----------------------|
| Compost                |            |                       |
|                        | +0         | 1.28 <sup>B</sup>     |
|                        | +1         | 1.39 <sup>A</sup>     |
| Stress                 |            |                       |
|                        | Control    | 1.31 <sup>A</sup>     |
|                        | Drought    | 1.42 <sup>A</sup>     |
|                        | Freeze     | 1.42 <sup>A</sup>     |
|                        | Heat       | 1.08 <sup>B</sup>     |
|                        | Glyphosate | 1.42 <sup>A</sup>     |
| Cropping system*stress |            |                       |
| C                      | Control    | 1.28 <sup>AB</sup>    |
|                        | Drought    | 1.53 <sup>A</sup>     |
|                        | Freeze     | 1.44 <sup>A</sup>     |
|                        | Heat       | 0.95 <sup>B</sup>     |
|                        | Glyphosate | 1.45 <sup>A</sup>     |
| O                      | Control    | 1.35 <sup>A</sup>     |
|                        | Drought    | 1.32 <sup>A</sup>     |
|                        | Freeze     | 1.41 <sup>A</sup>     |
|                        | Heat       | 1.21 <sup>AB</sup>    |
|                        | Glyphosate | 1.40 <sup>A</sup>     |
| <b>p-values</b>        |            |                       |
| Compost                |            | 0.015                 |
| Stress                 |            | <0.001                |
| Cropping system*stress |            | 0.025                 |



**Fig. 1** Microbial biomass was more resistant in organic than conventional in the main effect of cropping systems ( $p=0.017$ ).



**Fig. 2** There was no difference in resilience to glyphosate between cropping systems on DAS 7, 14, or 28. On DAS 2, conventional soils had greater resilience to glyphosate than organically managed soils ( $p=0.05$ ).



**Fig. 3** There was no difference in microbial resilience to heat stress between cropping systems on days 2, 14, and 28. However on day 7, conventional soils had greater resilience than organically managed soils ( $p=0.003$ ).

## CHAPTER VII: CONCLUSIONS

Quinoa's history as a successful crop in diverse ecosystems suggests the potential exists for the future climate of the western United States. However, the varieties in these studies proved poorly adapted to the field conditions in two typical growing seasons as there was very limited seed production. Before wide scale adoption is possible, factors influencing seed set must be resolved.

Relying on cover crop incorporation as the sole source of nutrients for a cash crop may provide adequate N additions, yet cannot provide complete nutrients, as demonstrated in our P deficient organic cropping system. Although seed yield was limited to only one season in the organic cropping systems trial, biomass growth responded positively to the addition of compost, likely due to a direct response to available P. The addition of extra N in the strip cropped system increased seed yield per row but reduced the effective cropping area by almost half. Compost and the relay- and inter-cropped systems showed increases in soil microbial measures in the course of only two growing seasons. A long term study of changes in the soil ecosystem function on nutrient cycling and soil health in these cropping systems would be beneficial.

Drought tolerant crops are also a key to farm sustainability throughout the region. Quinoa demonstrated varietal differences to drought conditions, however no seed was produced at any irrigation rate indicating available water was not the driving force in our systems. Further research into the water requirements of varieties that can reliably set seed under local conditions is still needed.

Under greenhouse conditions, quinoa proved to be competitive with three



common weed species. Increasing N applications increased both quinoa biomass and N accumulation. In general, quinoa was unaffected by the increase in weed presence at different planting ratios with both lambsquarters and redroot pigweed. Green foxtail was the most competitive with quinoa in both biomass yield and tissue N measures. The timing of emergence between species likely provided an advantage to quinoa over lambsquarters and redroot pigweed while green foxtail was similar to quinoa. As observed in the organic cropping systems study, the timing of quinoa emergence and fertility levels greatly influenced the weed pressure, and hence growth measures. Further study under field conditions with various fertility levels and cropping system management strategies are needed to fully describe the competitive abilities of quinoa.

The organic management of soil increased MB measures and the resistance to common agricultural stressors, with little response to additional compost inputs. The addition of compost also increased MB in conventionally managed soils. This suggests a history of diverse crop rotations and organic matter inputs both can increase critical soil health indicators and increase farm sustainability. The recovery of soils following stress events was likely influenced by the structure and degree of diversity in microbial populations as well as the available nutrients. Future work focusing on changes in community composition and the mineralization of organic matter following stress events could provide useful insights into driving factors of resistance and resilience.

In the future, maintaining farm sustainability will require locally adapted varieties, diverse cropping systems, and increased focus on soil health. If quinoa can play a role in these systems, it must have reliable seed set under the diverse temperature and irrigation conditions of our region. The benefits of diverse cropping systems and compost

applications were evident in soil health measures, nutrient availability, and resistance to imposed stress. Future work to examine the long term implications of cropping systems on soil health and crop production in the region are essential to support sustainable systems in the future.

## KRISTINE BUCKLAND

### Curriculum Vitae

#### EDUCATION

- Ph.D. in Plant Science 2016  
 Utah State University, Logan, UT  
 Dissertation: “Increasing the Sustainability of Utah Farms Through Incorporating Quinoa as a Novel Crop and Protecting Soil Organic Matter”
- M.S. in Plant Science 2011  
 Utah State University, Logan, UT  
 Thesis: “Evaluating Fertilizer Rate, Crop Rotation and Trap Crops for Effects on Onion Growth and Yield, Soil Health, Thrips Densities and *Iris Yellow Spot Virus* Incidence”
- B.S. in Aeronautical Engineering 1998  
 United States Air Force Academy, Colorado Springs, CO

#### RESEARCH INTERESTS

Sustainable agriculture  
 Crop protection through soil health and diverse cropping systems  
 Nutrient cycling and microbial community function  
 Agricultural systems analysis

#### RESEARCH EXPERIENCE

- Graduate Student/Research Assistant 2013-2016  
 Developed an organic quinoa production systems trial to examine integrating quinoa as a novel crop and cropping systems impacts on quinoa growth and soil health indicators. Implemented a line source irrigation trial to measure the varietal response of available quinoa varieties to a wide range of soil moisture. Designed and conducted two independent studies to address questions and concerns raised by the field trials. Evaluated weed competition of quinoa in response to weed species identity and density in response to nutrient inputs in the greenhouse. Constructed a laboratory trial to examine both conventional and organic soil resistance and resilience response as a function of prior management history. Quinoa variety trials were also grown on grower-cooperator fields over two years which involved coordinating available plots, seeding, maintaining the crops and data collection. Varieties were screened for salinity tolerance in the greenhouse for further incorporation in a replicated study.
- Graduate Student/Research Assistant 2009-2011  
 Accomplished a whole farm assessment to identify management strategies to reduce thrips populations and IYSV spread in onion including field management, data collection and analysis. Extensive field and laboratory work on chemical and

biological indicators of soil health as well as plant growth measurements to examine the impact of prior crop rotation and nitrogen fertilizer rates on onion growth and yield, soil health indicators, thrips pressure and disease incidence. Coordinated directly with local commercial growers over two field seasons for on-farm trial of trap crops in onions.

#### TEACHING EXPERIENCE

Research Mentor, Utah State University 2013-2015

Taught and mentored 12 undergraduate students over two field seasons. Trained students on the basics of field research including data collection and plant processing techniques. Developed three laboratory protocols for soil health measurements and successfully trained students to accomplish these intricate processes.

Evaluator Pilot, United States Air Force 2008-2009

Evaluating student performance as well as other instructors. Identified weaknesses in the current training programs and devise new ways to address these weaknesses. Developed an aerial video to help guide instructor and student pilots on ways to adapt their flight path in response to a challenging disruption on one of the airport's runways.

Instructor Pilot, United States Air Force 2004-2009

Taught over 100 young Air Force officers how to fly. Subjects ranged from basic aircraft maneuvers to multiple aircraft formation flights. Student evaluations accomplished by independent flight evaluators, with a greater than 90% of my students receiving passing evaluations. Produced capable pilots at rate greater than 95%, with zero safety related incidents.

#### SERVICE AND EXTENSION EXPERIENCE

Presenter and organizer of Annual Utah Quinoa Growers Meeting 2014

Held an annual meeting with local grower-cooperators and researchers to assess progress of field trials and prepare for upcoming field season

Presenter and organizer of the USU Quinoa Field Day 2014

This encompassed tours of field trials, presentations from regional quinoa experts and in-depth discussion of regional organic cropping systems.

Presenter at Utah Onion Grower's Association Field Day 2010

Presented poster with initial findings of replicated field trial on nitrogen fertilizer rates, crop rotation, and pest pressure

Presenter at Kaysville Agricultural Experiment Station Field Day 2010

Presented replicated field plots and initial findings to growers, extension agents, researchers and local community members

## PUBLICATIONS AND PAPERS

### Peer Reviewed Journals

Buckland, K.R., J.R. Reeve, D.G. Alston, D. Drost, and C. Nischwitz. "Effects of nitrogen fertility and crop rotation on onion growth and yield, thrips densities, Iris yellow spot virus and soil properties." *Agriculture, Ecosystems & Environment* 177 (2013): 63-74

### Presentations

Buckland, K.R., J.R. Reeve, D. Alston, D. Drost, and C. Nischwitz (December, 2009). "Evaluating the effect of nitrogen, crop rotation and trap crops on onion thrips, Iris Yellow Spot Virus and crop yield" *Presentation at annual W-1008 Multistate Research Project, Biology and Management of Iris Yellow Spot Virus and thrips in onion, San Antonio, TX.*

Buckland, K.R., J.R. Reeve, D. Alston, D. Drost, and C. Nischwitz (August, 2010). "Evaluating the effect of nitrogen, crop rotation and trap crops on onion thrips, Iris Yellow Spot Virus and crop yield" *Poster presented at the annual American Society for Horticultural Science conference, Palm Desert, CA.*

Buckland, K. R., "Evaluating Fertilizer Rate, Crop Rotation and Trap Crops for Effects on Onion Growth and Yield, Soil Health, Thrips Densities and Iris Yellow Spot Virus Incidence" (2011). *All Graduate Theses and Dissertations*. Paper 980.  
<http://digitalcommons.usu.edu/etd/980>

Buckland, K. R., J.R. Reeve, and J.E. Creech (November 2014). "Developing organic quinoa cropping systems for the Western United States." *Poster presented at the annual American Society of Agronomy conference, Long Beach, CA.*

Buckland, K.R., J.R. Reeve, and J.E. Creech (November 2015). "Developing organic quinoa cropping systems for Utah." *Presentation at annual American Society of Agronomy conference, Minneapolis, MN.*

## AWARDS

|   |               |
|---|---------------|
| Apogee Instrument-Campbell Scientific Graduate Fellowship                     | 2015          |
| Devere McCallister Scholarship  | 2015          |
| ASA/SSSA/CSSA Mott Award, Utah State University Nominee                       | 2015          |
| Elva Acklam and Avril Stark Scholarship, Utah State University                | 2014 and 2015 |
| Distinguished Graduate, Undergraduate Pilot Training, United States Air Force | 2000          |

## MEMBERSHIPS

ASA/SSA/CSSA