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Pre-Columbian Cultivation of Agave Species Through Rock Mulching: Potential for Modern Applications

Hector Genaro Ortiz Cano
Brigham Young University

Pre-Columbian Cultivation of *Agave* Species Through Rock Mulching:

Potential for Modern Applications

Hector Genaro Ortiz-Cano

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J. Ryan Stewart, Chair
Steven L. Petersen
Neil C. Hansen
Michael T. Searcy
Antonio Villanueva-Morales

Department of Plant and Wildlife Sciences

Brigham Young University

ABSTRACT

Pre-Columbian Cultivation of *Agave* Species Through Rock Mulching:
Potential Application for Modern Cultivation

Hector Genaro Ortiz-Cano
Department of Plant and Wildlife Sciences, BYU
Doctor of Philosophy

As global temperatures rise, cultivation of C_3 and C_4 crops in arid and semi-arid regions will face major challenges in producing biomass for billions of people. Conventional agricultural techniques that require copious irrigation will need to be complemented with dryland-farming techniques and drought-tolerant crops, such as those from the *Agave* genus, which use CAM photosynthesis. In the past and present, humans from arid and semi-arid regions of America have maintained a symbiotic relationship using and cultivating *Agave* (Agavoideae, Asparagaceae). In pre-Columbian times, Native Americans from arid regions relied on *Agave* cultivation as a subsistence crop to produce food, medicine, and fiber. The Hohokam in the Sonoran Desert cultivated *Agave* plants using rock mulching, also known as rock piles. This technique enabled the Hohokam to extensively cultivate *Agave* despite the limited rainwater available in the harsh Sonoran Desert. Although there are several decades of archaeological research for documenting the history of rock piles and *Agave* in the region beginning in the late 1970s, few studies have addressed the modern application of rock piles to cultivate *Agave*. Our research employed a multidisciplinary approach to bridge the historic use of rock piles to cultivate *Agave* with the potential application of rock piles for modern cultivation. In addition to summarizing what is known about the archaeology of Hohokam rock piles, we compiled an extensive review of the literature available on the agroecology, physiology, and natural history of *Agave*. We described key aspects associated with the hydrology and physical properties of Hohokam rock piles that can bolster *Agave* CAM photosynthesis in dry regions. We found that the use of rock piles is a feasible means of cultivating *Agave* under hot and dry conditions in arid regions. In addition, we used an ecological niche modeling approach and field data from Hohokam rock-pile sites and current *Agave* fields to assess the potential environments where rock piles could be used to cultivate *Agave* plants in Arizona, USA and Sonora, Mexico. We also combined an experimental archaeology approach with experimental plant physiology where we surveyed Hohokam rock-pile fields at archaeological sites to collect information about the composition of rock piles. We then created a rock-pile field where we evaluated and observed the effects of rock piles on *Agave* CAM utilization, mainly nocturnal CO_2 uptake of *Agave*. Our results indicated that rock piles provide direct insulation to root systems, which indirectly benefited *Agave* carbon uptake and reduced temperature and drought stress. Although more agronomic research about rock pile use is needed, our research suggests that rock piles can be applied to cultivate *Agave* because of the physiological benefits provided such as increasing nocturnal total CO_2 uptake. In addition, the suitability of rock piles in the U.S borderlands indicates that rock piles can be applied beyond the regions where they were used by the Hohokam in pre-historic times.

Keywords: Hohokam rock piles, *Agave*, CAM photosynthesis, dryland farming, CO_2 uptake.

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CHAPTER 1

Pre-Columbian Rock Mulching as a Strategy for Modern *Agave* Cultivation in Arid Marginal Lands

Hector Ortiz-Cano[1], Jose Antonio Hernandez-Herrera[2], Neil C. Hansen[1], Steven L. Petersen[1], Michael T. Searcy[3], Ricardo Mata-Gonzalez[4], Teodoro Cervantes-Mendívil[5], Antonio Villanueva-Morales[6], Pilman Park[7], and J. Ryan Stewart[1]

[1]Brigham Young University, Department of Plant and Wildlife Sciences, Provo Utah, USA
[2]Universidad Autónoma Agraria Antonio Narro, Departamento de Recursos Naturales, Saltillo Coahuila, Mexico
[3]Brigham Young University, Department of Anthropology, Provo Utah, USA
[4]Oregon State University, Department of Animal and Rangeland Sciences, Corvallis Oregon, USA
[5]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Costa de Hermosillo, Sonora México
[6]Universidad Autónoma Chapingo, División de Ciencias Forestales, Departamento de Estadística, Matemática y Cómputo, Chapingo, Mexico
[7]Floriculture Research Division, National Institute of Horticultural and Herbal Sciences, Rural Development Administration, South Korea
Doctor of Philosophy

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ABSTRACT

Cultivation of C_3 and C_4 crops in semi-arid regions will be severely constrained as global temperatures rise. Consequently, alternative crops need to be sought out that adapt well to heat and drought and are productive despite limited access to water. Traits, such as crassulacean acid metabolism (CAM), enable economically important species such as those in the *Agave* genus adapt to drought and high temperatures. The succulence and high efficiency of agaves, which enables them to produce biomass with little water, underscores their feasibility as an alternative crop for semi-arid regions, such as the Sonoran Desert in the southwestern U.S. In this paper, we offer a review of the suitability for cultivation of agaves via dryland farming, particularly by rock mulching techniques used by pre-Columbian, Sonoran Desert farmers. This analysis dovetails with information also provided on the biological traits of *Agave* and its historical and present utilization. Pre-Columbian, Hohokam dryland farmers used rock mulching in the form of rock piles to cultivate agaves. Rock piles acted as a type of mulch to harvest rainfall and to retain soil moisture, which allowed the Hohokam to intensively cultivate agaves during multi-year droughts. Remains of Hohokam rock mulching for agave production can be found at archaeological sites in central Arizona, which provides evidence of the utility of dryland farming and ancient agricultural innovation to reconcile water scarcity in the region. Moreover, the use of rock piles likely bolstered *Agave* productivity in marginal lands. Although little is known of historic rock mulching to cultivate agaves and its biological implications on plant productivity we suggest its application as a dryland farming model could be a sustainable strategy in the U.S. Southwest.

INTRODUCTION

Increasingly limited access to water and elevating temperatures will continue to hamper productivity of conventional C_3 and C_4 crops in arid and semi-arid regions throughout the world in the coming decades (De Micco and Aronne, 2012; Zandalinas et al., 2018; Porter and Semenov, 2005). Contemporary agricultural challenges, limited water availability, and rising temperatures have constrained agriculture in dry regions throughout history (Ingram, 2010). Within the last 1,300 years, global warming has been associated with overexploitation of natural resources, increased urbanization, and accelerated agricultural development (Woodhouse et al., 2010). Models of changes in global temperature suggest that induced anthropogenic global warming became more frequent during the Medieval Warm Period, which occurred between 700 and 1300 CE (Bradley et al., 2003; Galloway, 1986; Hughes and Diaz, 1994; Stinchcomb et al., 2011). The Medieval Warm Period increased temperatures and reduced water levels of lakes and rivers throughout Europe, Asia, and North and South America (Chu et al., 2002; Helama et al., 2009; Sridhar et al., 2006; Van West and Dean, 2000; Woodhouse et al., 2010). In addition to the Medieval Warm Period, some have hypothesized that global temperatures rose and rainfall patterns progressively changed in arid regions during the pre-industrial period 800–1850 A.D. due to land-use changes for agriculture (e.g., conversion of forests and grasslands into cropland) (Galloway, 1986; Pongratz and Caldeira, 2012; Reick, et al., 2010).

After the Industrial Revolution, the use of fossil fuels and concomitant increases in CO_2 emissions accelerated climate change (Callendar, 1938; Lemonnier and Ainsworth, 2018; Neftel et al., 1985; Revelle and Suess, 1957), increasing global temperatures and the occurrence of droughts during the 20th century (Hansen et al., 1981; Smith et al., 2019; Solomon et al., 2010). At the end of the last century and beginning of the 21st century, globalized industrial development and creation of large urban centers resulted in cropland expansion in arid and semi-arid regions to meet increased food-production demands (Krausmann et al., 2013; Laurance et al., 2014). Conversely, relatively warmer temperatures in the early part of the 21st century, high evapotranspiration rates, erratic rainfall, and increasingly severe droughts have deleterious consequences to farmland by reducing crop yields, resulting in an increase of marginal lands (i.e., farmland and wildlands with limited access to irrigation water and depleted soil nutrients) (Schlaepfer et al., 2017). These increasingly warm and dry conditions in regions with limited resources suggest future edaphic, biological, and climatic constraints for cultivation of C_3 and C_4 crops. Such conditions increase the need for seeking, selecting, and cultivating drought-tolerant crops, such as those found in the succulent *Agave* genus, which cope with drought through nocturnal CO_2 fixation and CAM photosynthesis (Borland et al, 2009, 2015; Stewart, 2015).

Current challenges in dry regions to cultivate and produce food in hot and water-limited conditions bear similarity to those that native people faced long ago during severe droughts in what is now the U.S. Southwest (Ingram, 2010). Irrigation water has always been a naturally limited resource in arid regions (Troyo-Diéguez et al., 1990). For dry regions, there is a need for sustainable agricultural strategies to optimize crop yields and irrigation water (Troyo-Diéguez et al., 1990). To cope with scarce availability of water, innovative, indigenous dry-farming strategies were developed anciently to produce food during droughts (Lightfoot, 1996). These dryland farmers irrigated with rainfall runoff by optimizing rainwater catchment and rewetting the landscape using manmade stone features, such as rock terraces and rock mulch (Lightfoot, 1994, 1996; Wilken, 1972). The indelible signature left by the historic use of rock terraces and rock mulching can be seen in ancient and modern societies in dry regions throughout the world. For example, in the Negev Desert of the Middle East, the nomadic Nabateans, who settled in the region around 600-300 BCE, built and used rock terraces, check dams, and rock mulching to irrigate and catch rainfall water (Ashkenazi et al., 2012; Evenari et al., 1982; Lightfoot, 1994; Stager, 1976). At the apex of Nabatean civilization, such terraces became the main dry-farming technology to cultivate olives (*Olea europaea*), pomegranates (*Punica granathum*), and apples (*Malus domestica*) (Ynnilä, 2007). Similar examples can also be found in ancient civilizations throughout the deserts of Africa, Europe, and Asia (Biazin et al., 2012; Lightfoot, 1994; Wilken, 1972). In the ancient Americas, rock-farming techniques were used in a variety of cultures and time periods (Kennett, 2012; Marcus, 2006). In the Andean region, from the times of the Huarpa civilization to that of the Incan (200 BCE to 1400 CE), rock-wall terraces were heavily relied on to cultivate potatoes (*Solanum tuberosum*), quinoa (*Chenopodium quinoa*), and corn (*Zea mays*) (Chapagain and Raizada, 2017; Denevan, 2003). Mayans in southern Mesoamerica were very effective in cultivating corn using rock terraces (Fischbeck, 2001; Turner, 1976; Webb, Schwarcz, and Healy, 2004). In central and northern Mesoamerica, Aztecs cultivated marginal lands with corn and agaves in a system called milpas (Evans, 1990; Trombold, 2017; Zizumbo-Villarreal et al., 2012). Ancient Pacific Islanders used rock mulching to harvest rainwater and to cultivate perennial crops, such as taro (*Colocasia esculenta*), in land with limited access to water (Ladefoged et al., 2013; Stevenson et al., 1999; Wozniak, 1999). Pre-Columbian Hohokam people, which inhabited the deserts of the American Southwest, also cultivated drought-tolerant agaves in marginal lands using rock-mulching (Fish and Fish, 1990; Fish and Fish, 1992; Fish, Fish, Miksicek, and Madsen, 1985; Gasser and Kwiatkowski, 1991).

Among historic dry-farming examples in the U.S., Hohokam agricultural dryland systems in central and southern Arizona are key to understanding applications of dry farming for other arid regions affected by drought. The Hohokam mastered desert farming (Fish and Fish, 1992). Their dry-farming techniques were adapted and designed to produce food in extended droughts and in the harsh Sonoran Desert climate. They implemented rock-mulching to catch rainfall water and successfully cultivate agaves to feed thousands of desert dwellers during water scarcity periods (Fish and Fish, 1990). Rock mulching turned into the primary strategy to shore up food production during droughts. *Agave* was the main crop that allowed for unabated cultural, social, and economic development in the region (Fish, 2000). As in the prehistoric past, modern central and southern Arizona is a region constrained by the harsh Sonoran Desert climate. Here the applications of indigenous dry-farming agriculture, principally Hohokam rock mulching, opens the possibility for cultivating agaves in current and future droughts. It is our intent to portray *Agave* as a drought-tolerant crop, which can be cultivated through the application of rock mulching to harvest rainwater as a feasible and sustainable dry-land agriculture system for arid regions. The purpose of this paper is to summarize literature available on 1) studies on the ecophysiology of agaves under drought conditions, 2) dry farming using rock mulching to cultivate agaves, 3) use of agaves throughout history, and 4) the potential of rock mulching and *Agave* cultivation in future droughts.

Biological traits of agaves and drought tolerance

Nearly 75% of the continental biological diversity of the *Agave* genus can be found in Mexico and 13% in U.S. deserts (Garcia-Moya et al., 2011; Gentry, 1982). The *Agave* genus evolved biological and morphological traits that enable species to adapt to erratic, hot, and drought-changing conditions of arid regions (Silva-Montellano and Eguiarte, 2003). Morphological traits of agaves, such as their shallow root systems, distinct rosette shape, and curved leaves to maximize rainfall interception, evolved to efficiently use small amounts of atmospheric and soil moisture in water-limited environments (Martorell and Ezcurra, 2007). Such limited water and heat conditions negatively affect the physiological performance of domesticated C_3 and C_4 crops (Nobel and Jordan, 1983). Crassulacean acid metabolism (CAM) photosynthesis is the main biological trait that drives productivity of these plants in hot and water-scarce conditions (Borland et al., 2011; Lüttge, 2004). Photosynthesis of agaves relies on nocturnal stomatal opening and CO_2 gas exchange as a strategy to avoid high evapotranspiration rates and leaf water loss during daylight hours (Lüttge, 2004). Nocturnal CO_2 fixation is the primary trait agaves use to survive dry climates and to adapt to warm temperatures (Borland et al., 2009). In addition, above and belowground morphological traits (North and Nobel, 1991), such as leaf succulence, rain-hair roots, and fibrous root architecture enable agaves to adjust physiological processes to available soil-moisture levels and heat in the different seasons of dry regions (De Micco and Aronne, 2012).

Agaves are monocarpic plants with a long life cycle to maturation (Nobel, 1977). Differences in plant maturation can be observed within and between species, regions, cultivation practices, and degree of domestication (e.g., domesticated agaves, hybrids of agaves, or wild agaves) (Zizumbo-Villarreal et al., 2013). Generally, cultivated agaves require a few years or up to a decade to mature to flower, and typically more than a decade to mature to flower in the wild (Cervantes et al., 2007; Núñez et al., 2008).

Aboveground morphological traits of agaves (e.g., shape, size of leaves, and succulence) enable these plants to survive and adapt to deserts by providing protection and storing water in the leaf parenchyma (Cervantes et al., 2007; Núñez et al., 2008; Orians and Solbrig, 1977). Additionally, the rosette arrangement of the curved *Agave* leaves funnel rainwater to the plant and soil during the summer monsoon season, re-wetting their rhizomes and the soil in the root zone (Gentry, 1982). Furthermore, Martorell and Ezcurra (2007) hypothesized that the rosette trait can also trap atmospheric moisture in the form of dew and fog between leaves. The thick succulent leaves of agaves function as plant water storage for periods of scarce rain and soil moisture. Even after a period of several months, when soil moisture has reached the permanent wilting point, agaves will remain physiologically functional (Nobel, 2003).

The *Agave* root system is composed of shallow roots (mean root length: 8 to 20 cm) and rhizomes (Arizaga and Ezcurra, 2002; Bautista-Cruz et al., 2007; Nobel, 2003). Offset growth from rhizomes and aerial bulbils act as the main asexual propagation strategy of agaves. Such offsets can extend several meters from the plant in search of soil moisture (Gibson, 1996; Nobel, 2003). Shallow roots allow rapid soil moisture absorption from the soil surface, particularly from small amounts of moisture deposited after light rain events (North and Nobel, 1991). Fibrous *Agave* root systems maximize soil water absorption, particularly in well-drained sandy soils with limited capacity to retain moisture (Cervantes et al., 2007). In addition, in very dry soils, dehydration of suberized peridermal cells of mature *Agave* roots prevents water loss and desiccation of the root vascular system (North and Nobel, 1991). Additionally, these lignified roots anchor *Agave* plants to the soil. When rainfall occurs, water pulses from rain rehydrate *Agave* roots, which promotes emergence of new root hairs, thereby increasing hydraulic conductance of *Agave* root systems (Palta and Nobel, 1989). Rainwater stimulates growth of ephemeral root hairs, which are vital for rapid water uptake and replenishing of water in *Agave* leaves (Huang and Nobel, 1992; North and Nobel, 1991).

Wild and cultivated agaves flourish in arid environments and poor soils in marginal lands (Cervantes et al., 2007; Gentry, 1972; Núñez et al., 2008).. Edaphic requirements include sandy soils with good drainage, 60% gravel content, and deep water tables (Cervantes et al., 2007). Particularly sandy loam soils with low salinity contents are optimum for healthy establishment of agaves. Agaves can be found growing in rocky soils in which temperatures can reach 70°C (Gentry, 1972; Nobel, 1994). Agaves typically perform well in soils with low nutrient content. Nitrogen levels in soils range between 31-35 parts per million (ppm) and low P between 2.6-3.0 ppm for optimal growth of agaves (Cervantes et al., 2007). Ideal growing conditions for agaves can be found in low-elevation mesic areas on hillslopes. In the Sonoran Desert of northwestern Mexico and southern Arizona, optimum elevation ranges for *Agave* growth have been observed between 800–1200 m above sea level (Gentry, 1972; Hodgson et al., 2019; Nobel and Hartsock, 1986; Núñez et al., 2008; Parker et al., 2014). However, agaves can also be found in the coastal areas of the Sonoran Desert in Mexico (Cervantes et al., 2007; Gentry, 1972; Núñez et al., 2008). Ideal precipitation levels for agaves vary from tropical to dry regions (Nobel, 2003; Gentry, 1982). In arid regions, such as Sonora, Mexico and Arizona, USA, agaves can survive rainless seasons for several years (Nobel, 2003). Some regions with wild populations of *Agave* receive as little as 7 mm of rain and other regions receive as much as 762 mm or more of annual precipitation (Gentry, 1982; Gentry, 1972; Nobel, 1976).

Cultivation of agaves using rock-mulching by the Hohokam

Who were the Hohokam?

The Hohokam were pre-Columbian dryland farmers that established a flourishing civilization in what is now central and southern Arizona between 450-1500 C.E. (Fish and Fish, 2008). Hohokam agriculture was constrained by the hot, dry climate, and the wide expanse of marginal lands in the Sonoran Desert (Fish and Fish, 1990). Drought and water availability for agriculture acted as definitive factors that influenced innovation in the agricultural and cultural development of the Hohokam (Hunt et al., 2005; Rice, 1998). These two factors shaped

Hohokam irrigation and dryland agriculture in the desert, leading to the use of extensive irrigation-canal networks and rock mulching to cultivate agaves during periods of drought with erratic rainfall (Fish and Fish, 2014b; Woodbury, 1961). Such approaches can be compared with dryland-farming systems observed in other advanced, prehistoric indigenous societies of Mesoamerica and South America (Doolittle, 1995; Fish and Fish, 2008). The irrigation canals of the Hohokam were similar to highly engineered Andean and Aztec irrigation-canal systems in that they were a pivotal factor in their cultural development and were designed to efficiently irrigate large areas of farmland, which led to substantial food production (Armillas, 1948; Bennett, 1948; Mitchell, 1973).

Irrigated crops formed the basis of Hohokam civilization and its economy. Efficiently distributed irrigation water from rivers and canals allowed large settlements to develop along the main rivers in the Phoenix Basin (Doyel, 2007). However, recurrent droughts at the beginning of the second millennia CE triggered periods of unstable food production, which changed agricultural strategies and the crops they cultivated (Fish and Fish, 1990). The Hohokam shifted to using more dryland farming and reliance upon rainfall for crop irrigation, resulting in less canal irrigation in the region.

During this period of recurring droughts, agaves were adopted as a crop to compensate for yield deficits during water shortages and as a supplement to irrigated annual crops (Anderies et al., 2008; Fish and Fish, 1992, 2008). The Hohokam primarily used rock piles to cultivate agaves, which enhanced their productivity during drought periods in the Sonoran Desert (Fig. 1-1) (Fish and Fish, 2014b; Fish et al., 1985).

Dryland farming using rock mulching to cultivate agaves

In the American Southwest, as in pre-Hispanic northern Mexico, the Hohokam implemented dryland agriculture strategies to cultivate *Agave* to ensure food security during droughts in the Tucson Basin (Anderies et al., 2008; Fish et al., 1985). A model of pre-Hispanic *Agave* cultivation by Anderies et al., (2008) suggests that *Agave* dryland farming was likely a strategy implemented by pre-Columbian groups to cope with climates with drought that reduced corn yields in the Sonoran Desert. The Hohokam adopted upland dryland farming on hillslopes using rock piles, terraces, and check dams to cope with low precipitation in the region (Fish and Fish, 1992). These structures allowed for the efficient use of rainwater to irrigate downhill floodplain crops and riparian vegetation (Fish et al., 1990). Rock piles and terraces were used to harvest rainfall runoff and to cultivate agaves (Fish and Fish, 1992).

Historical remains of Hohokam rock piles and evidence of *Agave* cultivation and processing in roasting pits can be found at archaeological sites outside the Tucson, Arizona area in the Tortolita Mountains; the Salt Gila Basin; the community of Marana; Tonto National Forest; San Pedro Valley; and Tumamoc Hill Reserve (Fig. 1-2)(Adams and Adams, 1998; Crown, 1987; Masse, 1979; Ciolek-Torrello et al., 1997). One of the most representative Hohokam rock-pile fields, which was found in Marana and characterized by Fish and Fish (1992), consisted of at least 42,000 rock piles nested with 120,000 m^2 of terraces and check dams within an area of 500 ha. They calculated that the rock pile fields could have annually produced 102,000 *Agave* plants with an average yield of 40.8 Mg ha^{-1}. However, comparing average planting density (i.e., 1000-3000 plants ha^{-1}) of agaves (Cervantes et al., 2007; Núñez et al., 2008) in modern plantations in the Sonoran Desert in Mexico, with the rock-pile fields found in

Marana, Arizona suggests that *Agave* productivity in Arizona was possibly higher than previously calculated by Fish et al. (1985) (i.e., 102,000 *Agave* plants in 500 hectares). For example, between 1000-3000 *Agave angustifolia* plants ha^{-1} can be annually cultivated in Sonora, Mexico in grasslands with no irrigation (Cervantes et al., 2007). Similarly, McDaniel (1985) suggested a planting density of 2000-2500 of *Agave americana* plants ha^{-1} cultivated in grassland in southern Arizona. If the calculations of Cervantes-Mendivil et al. (2007) and McDaniel (1985) regarding *Agave* cultivation area and planting density are applied to the largest Hohokam rock-pile field in Marana, Arizona, the minimum planting density would be 1000 plants ha^{-1} for 500 ha of rock-pile fields. As such, the Hohokam potentially had the capacity to cultivate nearly 500,000 agaves, which is at least five times more plants than previously estimated by Fish et al. (1985). However, commercial modern *Agave* cultivation differs from the cultivation strategies of the Hohokam. This example is only used to highlight the productive potential of the land to cultivate agaves in the region.

Hohokam agaves

Agave plant remains in Hohokam rock-pile fields underscore the importance of rock mulch for modern cultivation of agaves in the region (Fish and Fish, 1990; Fish, 2000; Fish et al., 1985). Though agaves were no longer cultivated prior to the arrival of Europeans to the region, agaves still can be found growing in some rock-pile fields and archaeological sites in Arizona (Hodgson and Salywon, 2013; Hodgson et al., 2019). Minnis and Plog (1976) observed a relationship between the occurrence of wild *Agave parryi* plants with proximity and distribution of agaves growing at archaeological sites in the Apache-Sitgreaves National Forest, suggesting putative historic cultivation of this *Agave* species in central Arizona. Similarly, Parker et al. (2010) observed genetic differences between putative cultigens of *A. parryi* and wild *A. parryi* plants at archaeological sites in central Arizona in the Mogollon Rim. Recently, a taxon, which was named *Agave sanpedroensis*, was discovered growing only in a rock-pile field west of Tucson, which is likely a relic of Hohokam cultivation (Hodgson et al., 2019). Living plants and dried tissue of *Agave* at rock piles and roasting pits have been found in Hohokam rock-pile fields at archaeological sites in southern and central Arizona (Adams and Adams, 1998; Fish, 2000; Fish and Fish, 2014b; Fish et al., 1985; Parker et al., 2007).

Little is known about cultivation of agaves using rock piles outside of Arizona. Minnis et al. (2006) found little evidence of rock piles around the prehistoric archaeological site of Casas Grandes in Chihuahua, Mexico. However, ethnobotanists and archaeologists that visited archaeological sites near Casas Grandes in 2018 and 2019 found agaves growing in ancient rock piles and terraces, perhaps indicating ancient cultivation similar to that found in Arizona (W. Hodgson and M. Searcy, personal communication). In 2018, putative hybrids of *Agave palmeri* and *A. parryi* were found growing in a rock terrace in the Casas Grandes region in northern Mexico (W. Hodgson, personal communication). Likewise, in 2019, *Agave* hybrids, which were similar in appearance to those discovered in 2018 were growing in rock piles (M. Searcy, personal communication) (Fig. 1-3). These recent findings suggest that cultivation of agaves using rock structures was likely more widespread than originally assumed. Such discoveries offer new avenues of research in the use of rock piles for prehistoric *Agave* cultivation.

Traditional uses of Agave as food, beverages, and as other sub-products

Indigenous people in Mexico and the U.S. Southwest have used agaves as a source of carbohydrates and fiber for the past 10,000 years (Delgado-Lemus et al., 2014). Diversity in the *Agave* genus is largely concentrated in Mexico and the U.S. Southwest, and was cultivated mostly using dryland farming techniques, such as rock mulching using rainwater runoff (Lightfoot, 1994, 1996), widely throughout tropical and arid regions of Central and South America (Good-Avila et al., 2006). Beginning in pre-Columbian times, *Agave* was used for various purposes in what is now the U.S. Southwest, including beverages, ceremonial items, fiber-based products (e.g., clothing, footwear, containers, cordage, nets, etc.), food, medicine, and paint (Castetter et al., 1938). Over the span of several centuries, particularly during droughts, agaves were an important energy source that enriched the diets of indigenous people (Anderies et al., 2008; Evans, 1990; Fish and Fish, 1990; Fish et al., 1985).

The Hohokam were one of the few pre-Columbian indigenous groups in the U.S. Southwest that extensively used rock piles to cultivate agaves as a staple crop that ensured a reliable source of food, even during droughts (Dobyns, 1988; Fish and Fish, 1992; Fish et al., 1985). The Hohokam relied on the ability of these plants to concentrate sugars in stems and stalks through their long phenological cycle. Sugars in *Agave* are inulin-type polymers of fructose that concentrate in leaves, stems, and inflorescence stalks (Mancilla-Margalli and López, 2006; Urias-Silvas et al., 2008). However, removal of *Agave* stalks at the end of their life cycle induces sugar accumulation predominantly in the stem (Cervantes et al., 2007; Hodgson, 2001; Michel-Cuello et al., 2008). Inflorescence stalk emergence indicates plants have matured, and are ready to be harvested (Arizaga and Ezcurra, 2002).

Pre-Columbian indigenous people used roasting pits, also called earth ovens, to cook their food, but particularly to roast agaves (Cervantes et al., 2007; Fish et al., 1985; Perry and Flannery, 2007; Walton, 1977; Zizumbo-Villarreal et al., 2009). Roasting pits can reach temperatures between 150-200°C (Cervantes et al., 2007). Roasting *Agave* heads (or caudices) at this temperature enables thermal hydrolysis to break down carbohydrate polymers into sugar monomers, such as fructose and glucose, which are relatively easy to digest and ferment by yeast (Cervantes et al., 2007), making it possible to use agaves as a food source. Methods of cooking *Agave* heads using earth ovens share similarities (e.g. shape, diameter of 1.20–2.40 m, depth of 0.80–2.10 m) between indigenous groups across various regions, both in ancient and modern times (Cervantes et al., 2007; Towell and Lecón, 2010).

The practice of roasting agaves can be traced to its origins in pre-Columbian archaeological sites in central Arizona and northern Mexico (Fish et al., 1985). In Arizona, *Agave* roasting pits also can be found in rock pile-fields (Fish et al., 1992). These roasting pits attest to the ancient use of roasted agaves and their cultivation in rock piles in the region. In addition, several documents from the Spanish colonial period recorded historic uses of roasted agaves by natives. Early colonial Jesuits from Spain, in what is now northwestern Mexico, recorded that agaves were used for medicinal purposes and were roasted for food by the Opata people in the Sonoran Desert (Flores and Araiza, 2012; Gutiérrez-Coronado et al., 2007). Similarly, in what is now central Mexico in the mid-sixteenth century, colonial Spaniards documented medicinal uses of roasted *Agave* by the Aztec people in the ethnobotanical compendium Codex Florentino (Díaz et al., 1993; Williams, 1990). Uses of roasted and fermented agaves for food were also recorded in the Codex Azcatitlan and Codex Boturini (Morán, 2008).

Modern use of Agave as a food source

As indicated above, out of all the organs of agaves, the stem head produces the most edible biomass (Nobel, 2003). Inflorescences, leaves, and stalks can also be used for food and to feed cattle (*Bos primigenius taurus*), sheep (*Ovis aries*), and goats (*Capra aegagrus hircus*) (Gentry, 1972; Pinos-Rodríguez et al., 2006, 2008, 2009; Hartung, 2016; Mellado, 2016). Gentry (1972) indicated that inflorescences of some *Agave* species are edible. Moreover, Fuentes-Rodriguez (1997) and Gentry (1972) suggest that the raw leaves remaining after clipping leaves from *Agave* stems, commonly called jimado in Spanish, can be used to feed cattle. Pinos-Rodríguez et al. (2006) found that leaves, flowering stalk, and bagasse of *Agave salmiana* can be used as food and to increase body weight of sheep. In popular Mexican cuisine throughout the country, *Agave* leaves are also used to cover goat or lamb stew while being cooked in underground roasting pits.

Alcoholic spirits and drinks from agaves

Aguamiel, pulque, and mezcal constitute the main beverages produced from agaves (Stewart, 2015). In order to produce aguamiel, an emerging inflorescence is cut out of the stem head. *Agave* sap, which is rich in sugars, accumulates in the remaining basin. The sap juice is subsequently siphoned out of the basin and prepared as non-alcoholic drink known as aguamiel. Fermentation of aguamiel creates pulque (Rivas, 1991), a commonly consumed, mildly alcoholic beverage in rural areas of central and southern Mexico (Enríquez-Salazar et al., 2017).

It has been estimated that pulque made of *Agave mapisaga, A. americana, Agave atrovirens*, or *Agave salmiana*, was consumed in approximately 2000 B.C. (Escalante et al., 2016). Although distilled *Agave* spirits like Tequila or mezcals are very popular in modern times, artisanal crafting and consumption of pulque remains alive in some regions of central Mexico.

Although drinks, such as aguamiel and pulque, are still consumed in modern times, these beverages have been somewhat replaced by distilled alcoholic drinks made from distillation of fermented sap of agaves (Garay and Aurea, 2008). Distillation technologies, such as the use of copper alembic stills to distill alcohol, were adapted to produce *Agave* spirits in Mexico by the Spaniards in the 1500s (Gutiérrez-Coronado et al., 2007; Towell and Lecón, 2010). In combination with a wide array of distillation methods, the diversity of *Agave* species in the different regions and the various cooking and fermentation methods of *Agave* heads employed by tribes across Mexico enabled a rich diversification of *Agave* spirits throughout Mexico from various species (Walton, 1977).

Tequila is the most popular *Agave* spirit crafted in Mexico, and differs from commercial mezcal in that it is made exclusively from *Agave tequilana* var. Azul, which is also known as blue agave (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2006; Vargas-Ponce et al., 2007). In contrast, mezcals are made from a wide diversity of agaves across Mexico. In addition, similar to some French wines, tequila has an appellation of origin (denominación de origen), which requires that blue *Agave* plants only be grown in certain states of Mexico that are believed to enhance the quality of tequila (Bowen, 2015). Tequila is mainly produced at an industrial scale following quality-control regulations compliant with national and international standards established by the Tequila Regulatory Council (Macías, 2001).

Despite climate change and political and economic changes throughout Mexican history, the tequila industry has experienced continual growth. For example, Walton (1977) reported 1.67 million liters of tequila were produced in 1960. Based on statistical data of total production of tequila from the Tequila Regulatory Council (2019), peak tequila production occurred in 2018 which coincided the highest production level ever reached over the past 23 years. In 1995, 104.3 million liters of tequila were produced, but increased to 309.1 million liters in 2018. Similarly, the Tequila Regulatory Council (2019) recorded that global consumption of tequila increased from 279 thousand tons in 1995 to 1,139 thousand tons in 2018. The Secretariat of Agriculture Livestock, Rural Development, Fisheries and Food (SAGARPA) in Mexico reported in 2017 that tequila, relative to mezcal, is the major product from *Agave* in an expansive growth phase in Mexico. Outside of Mexico, tequila is consumed mainly in the United States, Germany, Spain, France, and the United Kingdom. In addition, SAGARPA (2017) reported that the tequila industry generated approximately $27 million dollars from export revenue, which is predicted to increase to $28 million dollars by 2024 and $29 million by 2030. The mezcal industry has also experienced sustained growth from about 2.5 million liters in 1950 to about 20 million liters in 2010 (Martínez Salvador et al., 2012).

Sweeteners and syrups

Fructose sugars extracted from blue agave have also been used as alternative sweeteners (Heyer and Crawford, 2009; Stewart, 2015). Despite disadvantages of a relatively long life cycle and the monocarpic habit of agaves compared with other annual and perennial crops used in the sugar industry, *Agave* sugars are used as high-quality sweeteners. This emerging product can potentially work as a companion to the tequila industry. As with *Agave* spirits, the sweetener industry uses *Agave* juices as feedstock (Heyer and Crawford, 2009; Narváez-Zapata and Sánchez-Teyer, 2010). Sugars from *Agave* juice, particularly fructose, are extracted through acid or enzymatic hydrolysis (Ávila-Fernández et al., 2011; Garcia-Aguirre et al., 2009; Soto et al., 2011). The fructose sugars are used as additives in commercial *Agave* syrups, which are considered healthier sweeteners compared with sugar cane and high-fructose corn syrup (Hooshmand et al., 2014). The proportion of fructose in *Agave* syrup is significantly higher compared with the proportions in cane sugar and high-fructose corn syrup. Proportions of fructose to glucose are 50/50 in cane sugar (Glasziou, 1961); 55/45 in high fructose corn syrup (O'Brien-Nabors, 2001); and as high as 95/5 in *Agave* syrup (Garcia-Aguirre et al., 2009).

Modern uses of Agave fibers

Historically, *Agave sisalana, Agave fourcroydes* and *Agave lechuguilla* fibers have been used in the Mexican textile industry. Traditional uses of *Agave* fiber include ropes, twine, bags, mecapales, fabrics, brushes, and brooms (Colunga-GarcíaMarín and May-Pat, 1993; Kicińska-Jakuboska et al., 2012). Sisal is a hard fiber processed from the leaves of *A. sisalana*. Henequen fiber from *A. fourcroydes* and Tampico fiber (also called Mexican fiber) from *A. lechuguilla* have similar tensile and flexural properties as sisal fiber (Belmares et al., 1981; Kicińska-

Jakuboska et al., 2012). More recently, *Agave* fibers have been used to reinforce industrial products, adding flexibility and strength to polymer-based composites (de Andrade de Silva et al., 2010; Joseph et al., 1999; Orue et al., 2015). Fiber-reinforced polymers have application in the aerospace, marine, automotive, military, and construction industries (Yilmaz and Khan, 2019). Compounds derived from *Agav*e fibers, can also be used in synthetic drug manufacturing (Cushman et al., 2015). Steroidal saponins, tigogenin, and hecogenin are natural compounds extracted from *A. sisalana* leaves, which are used in the synthesis of steroidal hormones such as corticosteroids (Cripps and Blunden, 1978; Santos and Branco, 2014). Corticosteroids drugs like dexamethasone can be synthesized from tigogenin and hecogenin (Kongkathip et al., 1997; Santos et al., 2014) and may have application in treating respiratory-inflammatory conditions associated with COVID-19 produced by 2 SARS-CoV-2 (McIntosh, 2020; Saleh et al., 2020; Zhang et al., 2020).

Potential uses of agaves for bioenergy

Agaves have also been proposed as a biofuel crop due to their relatively low lignin content (Davis et al., 2011; Somerville et al., 2010). The high lignin content of C_3 and C_4 biofuel crops reduces the efficiency of converting sugars into bioethanol (Somerville et al., 2010). Lignin percentages in agaves range between 3-15% (Delfín-Ruíz et al., 2019; Iñiguez-Covarrubias et al., 2001; Li et al., 2012), and can be more efficiently processed to produce sugars than C_4 crops used in the biofuel industry (Somerville et al., 2010).

Many crops used in the biofuel industry require high amounts of irrigation water, generating controversy related to their environmental footprint (Moore et al., 2013; Somerville, 2007). Agaves require much less water than C_4 crops, such as corn, to produce biomass, and the superior quality of ethanol derived from *Agave* compared with corn makes agaves an attractive alternative (Yan et al., 2011). In addition to the low lignin content of *Agave*, CAM metabolism enables their growth in marginal lands and resilience to drought (Davis et al., 2011).

One challenge in the widespread use of agaves in the biofuel industry is the lack of agronomic knowledge for its cultivation, as well as the underlying ecology and climatic conditions of regions where this crop may be suitable (McDaniel, 1985). Lewis et al. (2015) suggested the U.S. Southwest, particularly Arizona, as one region for *Agave* cultivation for the biofuel industry, due to its suitable climate. Another constraint on *Agave* production involves the large amount of annual plant biomass needed to supply enough raw material to make it profitably sustainable as a bioenergy crop (Balan, 2014).

According to Escamilla-Treviño (2012), commercial cultivation of agaves for biofuel in the U.S. has been constrained mainly by the risk of low-temperature crop damage. For example, cultivation of species with a frost tolerance between -2 and -4°C, such as *A. tequilana*, *A. fourcroydes*, *A. angustifolia*, *A. salmiana*, and *A. sisalana* (Nobel, 2003), could be limited even in Arizona, where nocturnal low temperatures below 0°C occur throughout the winter season. Agaves could be genetically engineered to improve traits, which would allow for better adaptation from temperate to xeric environments, which would enable agaves to be widely cultivated in marginal environments in the U.S. (Yang et al., 2016). Another approach could be to use *Agave* species that have relatively wide cold tolerance, such as *A. americana* and *Agave utahensis*, which have been reported to adapt well to cold and hot temperatures in the region and can tolerate temperatures between -8 and -11°C (Davis et al., 2017; Escamilla-Treviño, 2012; Nobel and Jordan, 1983). Moreover, species putatively cultivated by the Hohokam in pre-Colombian times, including *A. palmeri*, *A. murpheyi*, *A. parryi*, and *A. sanpedroensis*, could potentially be used as crops in the future because they are endemic and well-adapted to hot summers, cold winters, and the dry climate of the Sonoran Desert (Adams and Adams, 1998; Fish and Fish, 2014a; Hodgson and Salywon, 2013; Hodgson et al., 2019; Parker et al., 2010).

Rock piles to cultivate agaves in marginal lands

Agave rock-pile fields are a cultural and agricultural legacy of the ancient Hohokam tribe in the Sonoran Desert (Hodgson et al., 2019). Rock piles are specialized features associated with the ancient practice of *Agave* cultivation in marginal lands (Dobyns, 1988; Fish and Fish, 1990; Fish and Fish, 2014a; Fish and Fish, 2014b; Sandor and Homburg, 2017). Because CAM metabolism enables agaves to grow well in water-limited environments and in nutrient-poor soils (Garcia-Moya et al., 2011; Gentry, 1982; Nobel, 1991, 2003; Nobel and Valenzuela, 1987), *Agave* was and is a well-suited crop for marginal lands. Prehistoric groups from central and southern Arizona lived in marginal lands with limited access to irrigation water (Fish and Fish, 1992). Rainfall was the primary source of water to irrigate agaves in rock piles. Rainwater in rock-pile fields was harvested in two ways: through the interception of rainfall hitting rocks and from rain-water runoff (Crown, 1987; Fish and Fish, 1992; Lightfoot, 1994, 1996).

Rock piles were built on downhill slopes such that the inclined angle mitigated downward runoff and enabled rock piles to reduce erosion and increase fertility of *Agave* fields (Fish and Fish, 1992; Sandor and Homburg, 2011). In such rock piles, rainwater generally flows along soil slopes, and upon intercepting rock piles, it slows down, leading to increased soil moisture beneath the rock piles (Fish and Fish, 1990; Fish and Fish, 1992; Homburg and Sandor, 2011). The reduced flow of rainwater leads to less gully formation. Likewise, sediments and minerals were mixed in the rainfall runoff and deposited underneath rocks. According to Homburg and Sandor (2011), the accumulation of minerals under rock piles improved the texture of the soil-surface horizons and increased soil moisture retention capacity. The minerals, sediments, and organic matter deposited below rock piles were a source of C, N, and P, which provided a source of soil fertility for agaves cultivated by ancient indigenous groups, such as the Hohokam.

Agave species likely cultivated by the Hohokam in rock piles

Evidence of cultivation of different *Agave* species in rock piles includes plant tissue, such as spines and fibers at nearby roasting pits and artifacts found in rock-pile fields, which were likely used to process and harvest agaves, such as tabular knives and scrapers (Cantley, 1991; Fish and Fish, 1992; Ciolek-Torrello et al., 1997). Among *Agave* species native to Arizona, *A. murpheyi* and *A. sanpedroensis* have been recognized as species that were cultivated in rock piles by the Hohokam (Adams and Adams, 1998; Hodgson et al., 2019; Parker et al., 2007). Moreover, researchers have identified *Agave yavapaiensis*, *Agave verdensis*, and *Agave delamateri* as pre-Columbian *Agave* cultigens (Hodgson and Salywon, 2013; Parker et al., 2007). Similarly, *A. parryi* has been associated with archaeological sites and dryland farming in Arizona (Minnis and Plog, 1976; Parker et al., 2014, 2010). Evidence of other cultivated plants used by the Hohokam include pollen grains of corn and cotton, which were found in rock piles (Bohrer, 1991; Crown, 1987; Fish, 1988). Other native species, such as *Opuntia spp*., *Carnegia gigantea*, *Chenopodium* spp., *Amaranthus* spp., *Trianthema portulacastrum*, *Spharalcea ambigua, Boerhaavia spp.*, and cholla (*Cylindropuntia fulgida*) have been found to populate rock-pile fields in archaeological sites (Bohrer, 1991; Crown, 1987; Fish et al., 1986; Hodgson et al., 2019).

Potential benefits of using rock piles to cultivate agaves

Rock-pile fields for agaves and their environments

While rock piles can be found at archaeological sites throughout the southwestern U.S. and northwestern Mexico, most are located in south-central Arizona (Fish and Fish, 1990; Fish and Fish, 1992; Fish et al., 1985), providing an ideal setting for modern *Agave* cultivation that could incorporate aspects of prehistoric rock-pile fields. Rainfall and temperatures at rock-pile fields in Marana, Tucson, and San Pedro Valley, Arizona, whose elevations range between 600–900 m above sea level, suggest that Hohokam agaves were cultivated in an optimum environment that balanced temperature, rainfall, and soil moisture (Cantley, 1991; Fish and Fish, 1990; Fish and Fish, 1992; Hodgson et al., 2019). This unique balance likely maximized productivity of agaves, even in the dry and harsh conditions of the region.

Different studies using the environmental productivity index developed by Nobel for agaves (Garcia-Moya et al., 2011; Nobel and Hartsock, 1986; Nobel and Quero 1986; Nobel and Valenzuela, 1987) found, in general, that mesic environments, such as archaeological sites with Hohokam rock piles, can lead to improved *Agave* productivity. The index indicates that CO_2 uptake and productivity of agaves is greater at elevations between 600–1200 m above sea level. In addition, Woodhouse et al. (1980) found that agaves are less productive when cultivated on steep slopes. Hohokam rock-pile fields occur more frequently on softly inclined slopes, which have higher rainfall moisture interception, and less negative soil water potentials than found on relatively steeper slopes (Cantley, 1991; Fish and Fish, 1992). Such conditions possibly promoted better interception of photosynthetic radiation and rainfall, which would have led to enhanced CAM photosynthesis and biomass of agaves. Understanding Hohokam rock pile field environments can help to identify potential locations to cultivate agaves, even during severe drought events. However, more information needs to be sought out to determine the agricultural limitations and future applications of rock piles in modern times.

Soil-water dynamics under rock piles

Hohokam rock piles functioned as a type of mulching that reduced soil evapotranspiration and used rainwater to increase soil moisture content underneath rocks (Doelle, 1978; Fish and Fish, 1990; Fish and Fish, 1992; Fish et al., 1985; Lightfoot, 1996). The positive effects of available moisture in agaves have been observed in different experiments. In an experiment with *A. deserti*, Jordan and Nobel (1979) observed that rainfall and soil moisture act as the most important factors influencing plant mortality in their first year of establishment. They also found that rainfall stimulated increased succulence, increased leaf growth, and helped modulate nocturnal CO_2 gas exchange and water-use efficiency of first-year plants. Davis et al. (2017) found that irrigation increased efficiency of nocturnal CO_2 uptake of *A. americana*. Similarly, Nobel et al. (1989) observed that irrigation doubled CO_2 uptake of *Agave lechuguilla* and enhanced aerial and root biomass. Lightfoot (1996) and Sandor and Homburg (2011) hypothesized that the moisture harvested underneath rock piles from rainwater improved cultivation of crops, including *Agave*, in rock piles (Fish and Fish, 2014a). In an experiment using agaves in rock piles conducted in different locations in central and southern Arizona, Fish and Fish (2014a) found that seasonal rains replenished soil moisture below rock piles, which improved survival rates of *A. murpheyi* and *A. americana*. Nobel et al. (1992) reported that after watering rocks with 10–30 mL of water, soil volumetric water content increased below rocks for a period ranging between 13–19 days, leading to increased nocturnal CO_2 uptake of *A. deserti*.

While moisture underneath rock piles likely enhanced *Agave* biomass productivity, Eickmeier and Adams (1978) indicated that available water and air temperature are the two most influential factors that affect *Agave* carbon assimilation. Although day-night temperature is an important factor in *Agave* nocturnal CO_2 uptake, as observed with *A. angustifolia* and *A. americana* (Holtum and Winter, 2014), soil moisture governs biomass productivity of agaves (Huang and Nobel, 1992). Nobel and Quero (1986) indicated that available soil moisture in summer and fall in the Sonoran and Chihuahuan Deserts acts as the driving factor that stimulates biomass of *Agave* plants by promoting emergence of new leaves, development of large aerial shoots, and enhancement of root hydraulic conductance. In addition, Nobel (1976) observed that leaf size and soil water content correlated with higher nocturnal CO_2 uptake of *A. deserti*. However, the benefits of increasing soil moisture by using rock piles needs to be further explored through additional lab and field experiments.

Soil temperature underneath rock piles

Cool temperatures below rock piles can reduce heat stress and desiccation of roots of *Agave* plants (Huang and Nobel, 1992). Since daily soil temperatures in the Sonoran Desert can reach 75°C (Nobel, 2003), rock piles can reduce soil-moisture evaporation rates (Sandor and Homburg, 2011), and also work as a barrier to reduce interception of solar radiation, which leads to cooler diurnal soil temperatures relative to that found in exposed soil (Wilken, 1972). Studies made on the thermal properties of rock piles in *A. deserti* and *A. americana* illustrate the advantages of rock piles as insulation to diurnal hot temperatures. Palta and Nobel (1989) observed that low soil temperatures underneath rocks positively affected *A. deserti* root respiration and reduced root dryness. Nobel et al. (1992) measured *A. deserti* roots underneath boulders or rock fragments and compared roots of agaves growing in exposed soils and found that low temperatures and less-negative soil water potentials underneath rocks increased root number, thickness, and length.

Kaseke et al. (2012) found that convective heat transference of rock mulch can keep soils cooler during the day and increase nocturnal soil temperatures. However, since little is known regarding patterns of diurnal and nocturnal temperatures underneath and within Hohokam rock piles, characterization of such properties is necessary in similarly arranged rock piles. In addition, experimentation is needed to characterize nocturnal convective heat transference underneath rock piles, and the level of nocturnal CO_2 uptake of agaves in rock piles.

Insulative properties of rocks and their effect on temperatures below rock piles likely have a positive effect on symbiosis of microbes with agaves (Cui and Nobel, 1992). A study on the effects of soil temperatures on vesicular-arbuscular mycorrhizae infection found that soil temperatures around 25°C increased yields of *Sorghum bicolor* and *Triticum aestivum* due to high colonization of roots by mycorrhizae (Fabig et al.,1989). Little is known, however, about the effect of Hohokam rock-pile temperatures on the soil microbiome and their associated benefits. Nevertheless, the *Agave* rhizosphere is diverse in prokaryotic and fungal microorganisms, and is correlated with the hosting capability of agaves and their adaptions to arid climates (Coleman-Derr et al., 2016). Symbiosis of *Agave* roots with soil microbes enhances root hydraulic conductance and nutrient uptake, particularly solubilizing P. Cui and Nobel (1992) observed that colonization of *A. deserti* with mycorrhizae improved hydraulic conductance, uptake of P in roots, and P allocation in leaves. In addition, colonization of mycorrhizae positively correlated with enhanced CO_2 uptake of *A. deserti*. Plant symbiosis with arbuscular mycorrhizae, ecto-mycorrhizae, ericoid mycorrhizae, and various bacteria contributes to increased uptake of N and P in the form of phosphates (Mensah et al., 2015). In a study where endophytic bacteria were isolated from the base of *A. tequilana* plants, Martínez- Rodríguez et al. (2014) identified the presence of 300 strains of bacteria with different capacities and benefits, such as N fixation, P solubilization, auxin production, and antagonism against *Fusarium oxysporum*.

Soil-based nutrients underneath rock piles

Agaves in the wild are well adapted to arid regions and generally perform adequately in rocky, nutrient-poor soils (Gentry, 1982; Gentry, 1972). However, soil-based nutrients underneath rock piles (Homburg and Sandor, 2011; Sandor and Homburg, 2017) can bolster *Agave* primary productivity (Nobel, García-Moya, and Quero, 1992). Soil research by Homburg and Sandor (2011) suggests that the use of Hohokam rock piles enriched C, N, and available P to agaves due to organic-matter accumulation. Soil nutrient accumulation underneath Hohokam rock piles possibly occurred due to runoff, microbial decomposition of organic matter, or soil bioturbation. The available nutrients below rock piles likely enhanced the physiological response of agaves to drought and extreme temperatures, and improved growth and productivity. However, it is necessary to assess the dynamics between soil-based nutrients underneath rock piles with *Agave* nutrient assimilation to more fully understand the benefits afforded by rock piles to *Agave* productivity. In addition, agricultural parameters, such as soil pH levels, soil electric conductivity, and nutrient cycling in the soil beneath rock piles, need future research.

Nutrients in the soil under rock piles, as observed by Homburg and Sandor (2011), contributed to the productivity of cultivated agaves. Available nutrients in the soil assist in the productivity of agaves (Nobel et al., 1992). Nobel et al. (1988) observed that fertilization with N, P, K, and B enhanced growth and nocturnal CO_2 uptake of *A. lechuguilla*. Similarly, irrigation, in combination with fertilization with N, P, and K, increased foliar leaf area, leaf number, and concentration of sugars, particularly fructose and glucose in *A. tequilana* and *Agave potatorum* (Martínez et al., 2012; Zúñiga-Estrada et al., 2018). Valenzuela and Gonzalez (1995) found that fertilization of *A. lechuguilla* and *A. tequilana* with P and N increased leaf area. Similarly, for *A. deserti*, Nobel et al. (1989) observed that fertilization promoted leaf growth and high CO_2 uptake, which led to high biomass accumulation.

Opportunity for researching agaves in rock piles

Relict rock piles at archaeological sites represent a valuable agricultural example of how the Hohokam made marginal lands productive by cultivating agaves during severe droughts. While a number of archaeologists reported that rock piles were the main dryland-farming strategy used by the Hohokam to cultivate agaves (Cantley, 1991; Crown, 1987; Dobyns, 1988; Fish and Fish, 1990; Fish and Fish, 1992; Fish et al., 1985; Lightfoot, 1996; Masse, 1979; Sandor and Homburg, 2011), little is known about the agronomic potential and applications of rock piles in modern *Agave* cultivation. To bring to light possible uses of rock piles, it is necessary to sort through what has been published regarding the environmental details of rock-pile fields in order to experimentally replicate these ancient agroecosystems.

Experiments that assess cultivation, pest management, and the physiological responses of agaves using rock piles are needed, particularly to observe plant productivity, CO_2 uptake, temperature requirements, and soil-plant water relations in rock piles. In addition, characterizing the hydrothermal properties of rock piles requires examining how they can preserve soil moisture and modulate soil temperatures. The microbiome and fauna of rock piles are additional factors that could potentially enhance nutrition, improve water status, and contribute to general plant health. Microbiomes in rock piles can positively impact plant health of agaves in future droughts, particularly in preventing pests and disease. Future research is needed on the environmental, social, and economic impacts of using rock piles to cultivate agave, particularly in the continually changing and fragile agroecosystems of dry regions.

CONCLUSIONS

Further experimentation and development of innovative agricultural strategies is crucial to the use of agaves as a crop under scenarios of severe drought and global warming. Throughout history agaves have been used as a commodity for food, drink, and fiber. In modern times, the use of agaves includes feedstock for sweeteners, biofuels, and synthetic drugs. *Agave* fibers are used to reinforce industrial materials. We suggest dryland farming of *Agave* as a means to minimize the use of irrigation water and sustainably maintain crop productivity in arid regions.

Current challenges to successful cultivation of agaves, such as low rainfall and excessive heat in arid regions and marginal lands, are similar to those that prehistoric, indigenous farmers faced during droughts in the Sonoran Desert. The pre-Columbian Hohokam were skilled in the use of rock piles to cultivate agaves during droughts (Dobyns, 1988; Fish and Fish, 1992). These rock piles acted as a mulch that harvested rainwater moisture, preserved soil moisture, reduced soil evapotranspiration, and insulated soil in their immediate environs. Despite the lack of empirical data, moisture harvested during the monsoon season beneath and around rock piles likely decreased drought stress, stimulating biomass productivity of agaves.

The use of rock piles for *Agave* cultivation promises ecological benefits, such as minimizing soil erosion and maximizing crop productivity in marginal lands with minimal input of chemical fertilization and pesticides.

Rock pile cultivation of agaves is promising, but it requires field-based research to characterize their productive potential. More research is also needed to understand how the Hohokam rock-pile system could be used to cultivate crops other than agaves. However, with even from the little that is known, rock piles provide a sustainable crop-production-technology alternative for efficient use of water in dry areas and to revive cultivation of agaves in limited-resource environments in the region.

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FIGURES

Fig. 1-1. Hohokam rock pile remains at Tumamoc Hill reserve in Tucson, Arizona.

Fig. 1-2. Archaeological site at Tumamoc Hill reserve in Tucson, Arizona.

Fig. 1-3. Agave growing in ancient rock pile at archaeological site in Casas Grandes, Chihuahua,

CHAPTER 2

Ecological-Niche Modeling Reveals Current Opportunities for *Agave* Dryland-Farming in Sonora, Mexico and Arizona, USA

Hector Genaro Ortiz-Cano[1], Jason Stetler[1], Teresa Gomez[1], Steven L. Petersen[1], Neil C. Hansen[1], Michael T. Searcy[2], Ricardo Mata-Gonzalez[3], Kevin Hultine[4], Teodoro Cervantes-Mendívil[5], David Burchfield[1], Robert Hadfield[1], Pilman Park[6], J. Ryan Stewart[1]

[1] Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah, USA
[2] Department of Anthropology, Brigham Young University, Provo, Utah, USA
[3] Department of Animal and Rangeland Sciences, Oregon State University, Corvallis Oregon, USA
[4]Department of Research, Conservation and Collections, Desert Botanical Garden, Phoenix Arizona, USA
[5]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Costa de Hermosillo, Sonora, México
[6]Floriculture Research Division, National Institute of Horticulture and Herbal Sciences, Rural Development Administration,
Jeonju, South Korea
Doctor of Philosophy

ABSTRACT

For centuries, humans occupying arid regions of North America have maintained an intricate relationship with *Agave* (Agavoideae, Asparagaceae). Today *Agave* cultivation, primarily for beverage production, provides an economic engine for rural communities throughout Mexico. Among known dryland-farming methods, the use of rock piles and cattle-grazed areas stand out as promising approaches for *Agave* cultivation. Identifying new cultivation areas to apply these approaches in Arizona, USA and Sonora, Mexico warrants a geographic assessment of areas outside the known ranges of rock piles and grasslands. The objective of this study was to predict areas for dryland-farming of *Agave* and develop models to identify potential areas for *Agave* cultivation. We used Maximum Entropy (MaxEnt) ecological-niche-modeling algorithms to predict suitable areas for *Agave* dryland farming. The model was parameterized using occurrence records of Hohokam rock piles in Arizona and grassland fields cultivated with *Agave* in Sonora. Ten environmental-predictor variables were used in the model, downloaded from the WorldClim climate database. The model identified potential locations for using rock piles as dryland-farming methods from south-central Arizona to northwestern Sonora. The *Agave*-grassland model indicated that regions from central to southern Sonora have the highest potential for cultivation of *Agave*, particularly for the species *Agave angustifolia.* Results suggest that there are many suitable areas where rock piles can be used to cultivate *Agave* in the Sonoran Desert, particularly in the border of southeastern Arizona and northwest Sonora. Likewise, cattle-grazing grasslands provide a viable environment for cultivating *Agave* in southern Sonora, where the expanding bacanora-beverage industry continues to grow and where different *Agave* products (e.g., syrups, fructans, saponins, and medicinal compounds) can potentially strengthen local economies.

INTRODUCTION

The deep-rooted symbiotic relationship between the *Agave* genus and indigenous groups and rural communities in arid regions has been crucial to creating sustainable agroecosystems in arid regions in Mexico and North America (Gentry, 1982; Fish & Fish, 1990). In the Sonoran Desert, beginning in pre-Columbian times, *Agave* was cultivated and used for centuries as an unabated source of food, drink, medicine, and fiber (Gentry, 1972; Fish & Fish, 1990, 1992; Bañuelos-Flores & Salido-Araiza, 2012). Desert farmers, such as the Hohokam, innovated dryland-farming techniques to cultivate *Agave* using rainfall runoff and rock mulching, also known as Hohokam rock piles (Masse, 1979; Fish & Fish, 1990, 1992; Homburg *et al.*, 2011;

Sandor & Homburg, 2015; Brevik *et al.*, 2016; Hodgson et al., 2019; Ortiz-Cano *et al.,* 2020). Hohokam dryland farming made the extensive cultivation of *Agave* possible as a staple crop in the Sonoran Desert (Fish & Fish, 1990, 1992). Principles of *Agave* dryland farming, which enabled the Hohokam to successfully cultivate *Agave*, can be applied to the modern *Agave* agricultural industry, which is currently impacted by rising global temperatures and droughts (Davis *et al.*, 2011, 2017, 2019; Ortiz-Cano *et al.*, 2020).

Agave cultivation in Hohokam rock piles ended before European contact (Dobyns, 1988). However, several indigenous groups continued using *Agave* for food and beverage after the demise of the Hohokam civilization (Dobyns, 1988; Bañuelos-Flores & Salido-Araiza, 2012). After the Hohokam, the Opata and Pima tribes continued using *Agave* as a crop*,* including through the use of roasting pits to cook *Agave* to craft beverages in the Sierra Madre Occidental Mountains (Rea, 1991; Cervantes-Mendívil *et al.*, 2007; Gutiérrez-Coronado, *et al.*, 2007; Bañuelos-Flores & Salido-Araiza, 2012; Domínguez-Arista, 2020). In the early 1900s, rustic distilled drinks made from *Agave angustifolia* in the Sierra Madre Occidental in Sonora, Mexico were identified by the name of mescal bacanora (Cervantes-Mendívil *et al.*, 2007; Domínguez-Arista, 2020). In the last 30 years, the bacanora industry and *A. angustifolia* as a crop have risen in economic prominence in the pueblos of the Sierra Madre Occidental, which can also be seen throughout the borderlands of Arizona, USA and Sonora (Salazar-Solano, 2007; Valenzuela-Zapata, 2009; Davis et al., 2011; 2019; Domínguez-Arista, 2020; Nabhan *et al.*, 2020). In 2019, the bacanora industry in Sonora produced 360,000 L of bacanora on 500 ha of land, with an estimated annual revenue of $3,392,640 (Integra, 2019). Current trends of land use for cultivating *A. angustifolia* for bacanora in Sonora has been estimated to expand 100 ha annually

(Integra, 2019). The European Union now recognizes mescal bacanora as a protected regional-based product of the state of Sonora (DOF, 2020). However, to continue cultivating *A. angustifolia* for bacanora and to promote *Agave* as crop in the borderlands of Sonora and Arizona, it is vital to find sustainable cultivation methods for *Agave* to thrive as the climate continues to change and to reduce the use of irrigation water in the rainfall-limited region (Nabhan *et al.*, 2020).

Increasing temperatures and droughts in the American Southwest will continue to constrain yields of irrigated C_3 and C_4 crops (Strzepek *et al.*, 2010; Clark *et al.*, 2016; Nabhan *et al.*, 2020). However, crops that use crassulacean acid metabolism (CAM) as their primary photosynthetic pathway, such as those in the *Agave* genus, are best suited to be cultivated in semi-arid and arid climates (Borland *et al.*, 2009; Stewart, 2015; Davis *et al.*, 2019). *Agave* cultivation using dryland-farming techniques, first implemented in pre-Columbian times, has great potential in the Sonoran Desert region, which straddles the border between Sonora and Arizona (Fish & Fish, 1992, 2014; Minnis, 2015; Nabhan *et al.*, 2020). However, producers of modern-day bacanora do not appear to be using dryland-farming techniques, such as rock mulching, for cultivating *Agave* in the Sonoran Desert region (McDaniel, 1985; Nuñez-Noriega *et al.*, 2008; Fragoso-Gadea, 2011). In addition, commercial *Agave* plantations do not currently exist in Arizona or other parts of the U.S. (Lewis *et al.*, 2015).

Agave cultivation in the borderlands of Sonora with Arizona, is in the early phases of development (Nabhan *et al.*, 2019, 2020). Northern-Sonoran *Agave* farmers are transitioning from a more traditional-rustic mescal industry to a more intensive and mechanized approach (i.e., use of tissue-culture facilities, greenhouses, and nurseries to propagate and produce plants; use of modern distillation equipment for mescal production) to produce mescal (Cervantes-Mendívil *et al.*, 2007; Nuñez-Noriega *et al.*, 2008; Esqueda-Valle *et al.,* 2016; Nabhan *et al.*, 2019, 2020). Two dryland-farming systems to cultivate *Agave*, rock-piles and cattle-grazing areas, show promise to offset the impacts of current climate challenges in the U.S.-Mexico borderlands region in the Sonoran Desert. *Agave* rock piles were used extensively by the Hohokam in Arizona to cultivate *Agave murpheyi* and *Agave sanpedroensis* (Adams & Adams, 1998; Fish & Fish, 1992, 2014; Hodgson et al., 2019). Rock piles modified the soil microtopography, increasing rainfall-water catchment, infiltration, and soil microflora, which favored water and nutrient uptake of agaves (Wilken, 1972; North & Nobel, 1991; Cui & Nobel, 1992; Lightfoot, 1994, 1996; Sandor & Homburg, 2015). In addition, rock piles acted as a barrier that protected *Agave* from natural predators (Pailes *et al.,* 2018). In more modern times, *Agave* has been successfully cultivated in cattle-grazing areas (commonly called agostaderos in Spanish) in rural areas of Sonora, such as grassland pastures, since the early 1900s (Cervantes Mendívil *et al.*, 2007; Fragoso-Gadea, 2011). Cattle-grazing areas with *A. angustifolia* are open areas with sandy-loam soils and medium-to-low stone content, in which shrubs, forbs, trees and native grasses are used for cattle foraging (Cervantes-Mendívil *et al.*, 2007; Esqueda-Valle *et al.,* 2016). In these areas, some species act as nurse plants, providing shelter and nutrients for *A. angustifolia* (Esqueda-Valle *et al.,* 2016). Using cattle-grazing areas enables Sonoran *Agave* farmers to diversify land use and income streams by raising livestock and *Agave* for producing bacanora. Incorporating *Agave* cultivation, which primary relies on rainfall instead of irrigation, would require only a minimum investment to implement (Fragoso-Gadea, 2011; Esqueda-Valle *et al.,* 2016). Nevertheless, despite the potential of these two dryland-farming systems, *Agave* cultivation in the Sonoran Desert is a notably under-utilized opportunity to expand the agricultural economies in the borderlands region of the U.S and Mexico (Nuñez-Noriega, 2004; Davis *et al.,* 2019).

Given the high degree of water scarcity in the borderlands region, coupled with a lack of infrastructure to establish and maintain irrigation systems, a clear need exists to identify potential areas suitable for *Agave* dryland farming. Forecasting areas for *Agave* dryland farming could clearly benefit our understanding of *Agave* as a viable crop and help to expand its cultivation in the region. Making informed decisions on the use of land and conservation in the Sonoran Desert requires an assessment of suitable areas for potential *Agave* cultivation. This assessment is crucial given that predicted heat waves and droughts will increase in frequency and duration throughout the borderlands in the coming decades (Seager *et al.*, 2007; Notaro *et al.*, 2010).

Potential areas for dryland farming can be identified through remote-sensing techniques and ecological- modeling platforms, which use suitability-modeling techniques for predicting areas based on known dryland-farming locations and environmental aspects of the region (e.g., temperature, elevation, slope, aspect) (Wei *et al.*, 2018). Field observations can document the occurrence of plant or animal species, environmental attributes, or agricultural features that can be effectively used in geographic-distribution models. This kind of approach offers a low-cost, first-approach option to evaluate suitable areas for *Agave* dryland-farming. In order to identify suitable climate and areas that potentially can support *Agave* dryland-farming in the region, we used the Maximum Entropy (MaxEnt) modeling platform (Phillips *et al.,* 2017) to identify areas that are suitable for *Agave* dryland cultivation in southern Arizona and Sonora. MaxEnt uses ecological-niche theory (Merow *et al.,* 2013) and maximum-entropy principles (Phillips *et al.,* 2005; Li & Guo, 2010) to create a probabilistic model of the occurrences and geographic extent of the species in a selected area using geographic locations and environmental information from a given area. We chose MaxEnt because it has been widely used in several research fields to create geographic-distribution models of animals, insects, wild plant species, crop species, and archaeological features (Phillips, 2005; Pearson *et al.*, 2007; Hoffman *et al.*, 2008; Ardestani *et al.*, 2015; Healy *et al.*, 2017; Phillips *et al.*, 2017; Elith *et al.*, 2019). In addition to modeling distributions of rock piles and cattle-grazed areas for potential *Agave* cultivation, MaxEnt also offers an accurate, simple, and information-rich analysis of environmental factors to forecast suitable climates to cultivate *Agave* using dryland farming (Healy *et al.*, 2017; Elith *et al.*, 2019). Forecasting potential suitable areas for *Agave* dryland farming is crucial for supporting local economies, which are reliant on agricultural production and distribution of *Agave* throughout the region (Nabhan *et al.*, 2019, 2020).

To identify new potential areas for *Agave* cultivation using dryland-farming techniques, we created suitability models using MaxEnt, which were based on existing Hohokam rock piles in Arizona and in cattle-grazed areas in Sonora where agaves were cultivated. We also used MaxEnt to evaluate if dryland-farming areas coincide with *Agave* natural habitats. We made models of 1) *Agave parryi,* a species endemic to northern Sonora and southern Arizona, which is not cultivated, but has potential to produce biofuel, phytochemicals, and mescal (Castetter *et al.,* 1938; Mielenz *et al.,* 2015), 2) *Agave palmeri,* which traditionally has been used to produce mescal in northern Sonora and studied for medicinal uses (Castetter *et al.,* 1938; Bahre & Bradbury, 1980; Mthembu & Motadi, 2014), and 3) *A. angustifolia,* which is extensively used to produce mescal within the protected designation-of-origin region for mescal bacanora in the Sierra Madre Occidental Mountains in Mexico. In developing these models, we had three main goals. First, assess the performance of MaxEnt modeling as a tool to predict suitable dryland-farming areas in Arizona and Sonora. Second, analyze individual models to identify potential areas based on their environmental suitability for *Agave* dryland farming. Third, compare models to determine which dryland-farming technique (i.e., rock piles or cattle-grazed cultivation) is most suitable for specific areas.

MATERIALS AND METHODS

The study area

We selected an area encompassing Arizona and Sonora and adjacent states in the borderlands of the U.S. Southwest and northwestern Mexico (Appendix 1). We included the states that share borders with Arizona, which comprise southeastern California, southern Utah, southern Nevada, and western New Mexico. In Mexico, we included eastern Chihuahua and northern Baja California.

MaxEnt species distribution modeling

We used MaxEnt software version 3.4.4. (Phillips *et al.*, 2017) to create distribution models for where *Agave* may have been cultivated in rock piles and grasslands and where there might be potential cultivation of relevant crop plants within the *Agave* genus. Developing distribution models in MaxEnt involves inputting known geographic locations where samples have been found and relevant environmental variables (e.g., rainfall, temperature, elevation, aspect, etc.) to produce a probability map of habitat suitability (Phillips *et al.*, 2017; Elith *et al.*, 2019). In the MaxEnt software, species, geographic location of localities, agricultural features, and plant or animal species can be processed with as little as three samples available in a known area (Proosdij *et al.*, 2016).

Dryland-farming occurrences: rock piles and Agave grassland samples

We used geographic information from two dryland-farming systems for *Agave* in this study: 1) Hohokam rock piles in Arizona and 2) grassland fields cultivated with *A. angustifolia* in Sonora. A global-positioning-system (GPS) unit (Oregon 600, Garmin, Olathe, KS, USA) was used to record the location of rock piles at archaeological sites in Arizona using Universal Transversal Mercator (UTM) WGS84 as a coordinate system. Forty-four Hohokam agave rock piles were sampled at archaeological rock-pile fields in southern and central Arizona. The selected sites were previously reported in the literature (Masse, 1979; Cantley, 1991; Fish & Fish, 1992, 2014; Whittlesey *et al.*, 1997) (Table 2-1). Field surveys were conducted between 2017 and 2019 with the assistance of Hohokam rock-pile experts, emeritus anthropologists Paul and Suzanne Fish, of the Arizona State Museum and Desert Laboratory on Tumamoc Hill.

We also identified geographic locations of grassland fields where *A. angustifolia* (Gentry, 1972) is cultivated through an inventory of wild and cultivated *Agave* species conducted in Sonora (INIFAP, 2011). This is the most recent inventory made of wild *A. angustifolia* species after the one made by Gentry (1972). The inventory includes *A. angustifolia* commercial plantations for bacanora in the Sierra Madre Occidental Mountains in Sonora (Cervantes-Mendívil *et al.*, 2007) (Table 2-1).

Agave sample occurrences

We selected *Agave parryi* (Castetter *et al.,* 1938; Gentry, 1972) due to its long association with pre-Columbian *Agave* cultivation at archaeological sites in east-central Arizona and its potential use for mescal in the borderlands of Sonora and Arizona (Castetter *et al.,* 1938; Minnis & Plog, 1976; Parker *et al.*, 2014; Mielenz *et al.,* 2015; Nabhan *et al.*, 2019). We also selected *Agave palmeri* (Castetter *et al.,* 1938; Gentry, 1972) because of its use in producing an artisanal mescal commonly called lechuguilla in northern Sonora (Castetter *et al.,* 1938; Bahre & Bradbury, 1980; Klopper *et al.*, 2010). Moreover, both species are endemic to the borderlands of Arizona and Sonora (Castetter *et al.,* 1938; Gentry, 1972, 1982; Bahre & Bradbury, 1980; Klopper *et al*., 2010; Nabhan *et al.,* 2019). We collected *A. parryi* and *A. palmeri* geographic location data from an online herbaria database (SEINet, 2020). In addition, geographic locations of wild *A. angustifolia* species were identified through an inventory conducted by INIFAP (2011).

Preprocessing of occurrences for MaxEnt modelling

Since the distance between rock-pile clusters at archaeological sites vary (Fish *et al.*, 1985; Fish & Fish, 1990), we used the 'thin' function of the spThin package to subsample rock piles from all the sites sampled (Aiello-Lammens *et al.*, 2015). We assessed autocorrelation between samples using the software RStudio Version 3.6.3 (R Development Core Team, 2020). The optimal-setting parameters for running MaxEnt were extracted using the ENMeval R package (Muscarella *et al.*, 2014) for each dryland-farming system and wild *Agave* population selected for the study to ensure generation of models with high predictive power. MaxEnt model settings were extracted from predictor variables and sample occurrences using ENMTools in RStudio (Warren *et al.*, 2010).

Environmental predictor variables

We downloaded environmental variables (Table 2-2) including slope, aspect, elevation, and solar radiation with 30-second (ca. 1 km) spatial resolution from WorldClim (www.worldclim.com/bioclim) version 2 datasets (Fick and Hijmans, 2017). We processed the variables using the 'extract by mass' tool in ArcGIS (version 2.4) to produce environmental layers. We selected climatic variables as predictors, taking into consideration that *Agave* productivity, physiological performance, and cultivation can be reliably predicted using temperature, precipitation, and solar radiation (Nobel, 2003; Garcia-Moya et al., 2011).

After variables were processed, we analyzed multicollinearity using cross-correlations (Pearson correlation coefficient *r*) using ENMTools in RStudio. To ensure minimal correlation between the most relevant environmental factors, also identified as predictor variables for distribution-suitability modeling, we used values <0.7 in the Pearson correlation analysis as suggested by Júnior and Nóbrega (2018) and Feng et al. (2019). Nine continuous environmental variables, which were included in our analysis, are summarized in Table 2-2. The MaxEnt analysis determines the level of importance, or the test gain, of each variable. The test gain is particularly important in analyzing the variables to explain model trends.

Model performance and validation

We used the cross-validation method (Radosavljevic & Anderson, 2014) to assess the model performance of the two dryland-farming systems. We analyzed the models with the 'leave-one-out' method, also known as jackknife analysis (Pearson *et al.*, 2007). The 'leave-one-out' method is a systematic approach that tests the individual contribution of each data point to the overall model performance. The method has been used in ecology and conservation biology when dealing with small size data sets, which have ≤ 25 samples. This method has been tested widely in different ecological-niche models to build species-distribution models of large areas with limited data occurrences (Pearson *et al.*, 2007; Baldwin, 2009; Proosdij *et al.*, 2016).

In addition, we used the area-under-the-curve (AUC) approach to validate the modeled dryland-farming and wild *Agave* population distributions, which were generated using MaxEnt. The AUC method estimates the ratio between true-positive rate and the false-positive rate, which allows for predicting and ranking locations using only presence data (Pearson *et al.*, 2007; Merow *et al.*, 2013). We used a binomial test to evaluate the prediction-potential accuracy of MaxEnt (Phillips, 2005; Pearson *et al.*, 2007; Elith *et al.*, 2019).

We evaluated the importance of environmental-predictor variables in the individual models. MaxEnt evaluates distribution models by tracking which environmental variables most influence the predicted distribution in the model (Phillips *et al.*, 2017). The MaxEnt algorithm uses the gain function to estimate the model predictivity likelihood. The gain function estimates the ratio between occurrences and predicted sites in the environmental variables selected to create the model (Merow *et al.*, 2013; Phillips *et al.*, 2017). We examined which variables contributed most to building prediction-distribution models of dryland-farming systems and for various *Agave* species. We then built response curves of the environmental predictor variables (Figure 2-4), which provided the most significant contributions to the model.

Comparisons of dryland-farming models with Agave species

In order to understand the relationship between the extent of dryland-farming systems and *Agave* species in the region, we paired models using the identity test in ENMTools (Warren *et al.*, 2010), also called permutation-analysis assessment, to statistically test if the predicted model distributions of dryland-farming systems and *Agave* species overlapped in the region. MaxEnt creates probability-habitat-suitability scores as functions of the environment across the landscape (Warren & Seifert, 2011). Likelihood of species similarities can be quantified in the distribution models by analyzing suitability scores of each individual pixel (e.g., spatial gain) in the MaxEnt output (Phillips *et al.*, 2017). We evaluated the estimated levels of closeness or habitat occupancy overlapping between species distribution in both dryland-farming systems and wild *Agave* species using the permuted *I*-statistic (permutation test) or identity test, which is widely used in understanding ecological-niche models (Walters *et al.*, 2017; Senula *et al.*, 2019). The *I*-statistic uses the occurrence data to randomly extract subsamples from MaxEnt maps and then reanalyzes the distribution to parameterize species and to calculate overlapping. This method allows researchers to quantify how similar or different the distributions are between two species, and how they are related with respect to their habitat predictability (Walters *et al.*, 2017). We considered significant differences between species-habitat distributions (non-identical niches) when the observed (non-permuted) *I*-statistic values were below the critical (permuted) threshold (5%) in ENMTools (Warren *et al.*, 2010; Walters *et al.*, 2017; Senula *et al.*, 2019).

Permutation analysis assesses the similarities of the predicted areas using MaxEnt modeling (Phillips, 2005; Pearson *et al.*, 2007). A permutation test or identity test is a comparative analysis of two probability distributions from two MaxEnt models to statistically evaluate how close these models are in their predicted geographic range (Warren *et al.*, 2010). Significant differences between models can be inferred when the observed value is lower than the critical value (Walters *et al.*, 2017; Senula *et al.*, 2019). While there are limited examples of using this kind of analysis for characterizing suitability models in agriculture (Reddy *et al.*, 2015 a, b; Wei *et al.*, 2018), this analysis can be used to understand the differences and similarities between the environments and to assess their dryland-farming suitability.

Software

The *Agave* dryland-farming and *Agave* population distribution models were created in the Java program MaxEnt 3.4.4 (Phillips, 2005). We used Dismo package (Hijmans *et al.*, 2016) and ENMTools to analyze model overlap, species occurrences, and species-distribution maps. We analyzed preprocessing of species occurrences and climatic layers using ENMeval (Muscarella *et al.*, 2014) and ENMTools in RStudio Version 3.6.3.

RESULTS

Model performance and validation

The models for the two dryland-farming systems (i.e., rock piles and *Agave* grasslands) and also for wild *Agave* species yielded AUC values between 0.96 and 0.99 (Table 2-3). Overall, the jackknife analysis indicated differences between variable importance for rock piles and *Agave* grasslands and between *Agave* species (Figure 2-1 and Table 2-3). For rock piles in

Arizona, the elevation range was the variable that contributed the most (64.6%) to build the distribution of dryland farming across the state and in the borderlands with Sonora. Suitability models for *Agave* grasslands in Sonora, indicated that the variable, precipitation seasonality, contributed the most (72.6%) to construct the model. *Agave parryi* had a similar association with precipitation and elevation as observed for rock piles, indicating precipitation seasonality and elevation as the two most influential environmental variables to the suitability model of this *Agave* species in the region (Table 2-1). Precipitation in the wettest quarter of the year (i.e., June, July, and August) was the main variable in predicting potential suitable areas for *A. angustifolia* in Sonora (Figure 2-3a, b). In contrast, the model of *A. palmeri* indicated that mean temperature in the wettest quarter was the main variable to predict suitable areas for this *Agave* species in the borderlands of Arizona and Sonora.

Suitable areas for dryland-farming and Agave cultivation

The rock-pile model suggested potential suitable areas for rock piles in central and southern Arizona, primarily in the southern borderlands of Arizona, including Pima County and the reservation lands of the Tohono O'odham (Figures 2-2a and b). In central Arizona, potential suitable areas span through Gila, Pinal, and Yavapai Counties. The rock-pile model yielded potential locations from south-central Arizona to northwestern Sonora. The *Agave*-grassland model (Figure 2c) indicated that the regions of central and southern Sonora have the highest potential to cultivate *Agave*, particularly *A. angustifolia*.

The locations with the highest suitability for *A. palmeri* and *A. parryi* in the Sonoran Desert are concentrated in the borderlands between Arizona and Sonora (Figures 2-3a and b).

However, the suitability of both species decreases towards southern Sonora. The model suggests that suitable areas to cultivate *A. angustifolia* in southern Sonora center in the Fuerte-Mayo region, while the northern mountain range of the Sierra Madre Occidental in Sonora in the northern boundary of the designation-of-origin region for bacanora is less suitable (Figure 2-3c) (Appendix 2-2).

Importance of environmental variables to predict suitable areas

Our results suggest that none of the individual variables showed similar test-gain values when compared with the full-model test-gain values (Table 2-4). This indicates that all variables are necessary to predict suitable areas for *Agave* species and potential dryland-farming areas in the region.

The most suitable areas to employ rock piles had elevations between 650-1200 m above sea level (Figure 2-4a). These areas also had precipitation seasonality values ranging between 40-70% (Figure 2-4b). Precipitation seasonality is an estimation of the deviation of the monthly variation of annual rainfall, which is also called coefficient of variation of annual precipitation, and is an index of inter-annual rainfall variability. The index describes fluctuations and likelihood of precipitation in a region. It is calculated as the ratio of the standard deviation of the monthly total precipitation to the mean monthly total precipitation expressed in percentage. The higher the percentage of this index, the higher variability of rainfall in a region. Estimated rainfall for suitable rock-pile areas was estimated at 100 mm with no more than 150 mm in the wettest season of the year, which is during the North American monsoon season (i.e., June, July, August) (Figure 2-4c). In addition, the most suitable areas to employ grasslands had elevations between 700-1300 m above sea level (Figure 2-4a) and precipitation around 300 mm during the summer months (Figure 2-4c), which accounts for more than 80% of the deviation of the monthly variation of the precipitation over the course of a year (Figure 2-4b).

The most suitable areas for wild *Agave* species had elevations between 200-1000 m above sea level for *A. angustifolia* and between 1400-1500 m above sea level for *Agave palmeri* and *Agave parryi* (Figure 2-4a). In addition, rainfall in suitable areas for *A. angustifolia* was estimated in areas with 80% of annual mean precipitation above 300 mm of rainfall. Suitable areas for *A. palmeri* are those that received around 200 mm of rainfall during the monsoon season. Suitable areas for *A. parryi* areas can be found where precipitation averages 250 mm of rain during the monsoon season (Figure 2-4 b,c).

Comparisons between dryland-farming systems and areas suitable for cultivation of Agave

Predicted distribution of rock piles and grasslands cultivated with A. angustifolia

The permutation test to compare distributions of rock piles with grasslands cultivated with *A. angustifolia* yielded 0.52 for the observed value and 0.47 for the critical value (Table 3). Slight differences between observed and critical values of rock-pile fields and grasslands models using the permutation test suggest that although distributions of the two dryland-farming systems were predicted in different areas (Figure 2), the differences between their predicted geographic ranges were indistinguishable using the permutation test.

Rock piles paired with Agave species

Rock piles paired with *A. palmeri* and *A. parryi* in the permutation test indicated significant differences in their geographic distributions (Table 2-5). Analysis of the proximity of predicted distributions of the rock-pile model with *A. palmeri,* using the permutation test, yielded an observed value of 0.39 and a critical value of 0.46. Distribution of rock piles compared with *A. parryi* distribution yielded an observed value of 0.21 and a critical value of 0.35. These results suggest that the predicted areas in the suitability models (Figures 2 and 3) occur in different areas within the borderlands of Arizona and Sonora.

Comparisons of Agave angustifolia with A. palmeri and A. parryi

Comparisons of *A. angustifolia* with *A. palmeri* and *A. angustifolia* with *A. parryi* indicate that the observed values were lower than the critical-value permutation scores (Table 2-5), suggesting that predicted suitable areas for *A. palmeri* and *A. parryi* species occur in areas different from those with *A. angustifolia*.

Cattle-grazed grasslands paired with A. angustifolia

Suitability models for grasslands and for *A. angustifolia* (Figure 2-2c and 2-3c) indicate that wild *A. angustifolia* overlaps with predicted grasslands areas. The analysis of *A. angustifolia* with *Agave* grasslands, which yielded an observed value of 0.46 and a critical value of 0.44, did not show differences in their distributions, suggesting that they occur in similar areas in Sonora. In southern Sonora, we found that there was considerable overlap between grassland areas and predicted suitable areas of wild *A. angustifolia* (Figure 2-3c).

DISCUSSION

Predictability of MaxEnt dryland-farming models

We found that MaxEnt is a powerful tool for modeling suitable areas for *Agave* dryland farming, particularly using rock piles, as Healy *et al.* (2017) used for suitability of Ak-Chin dryland features. MaxEnt models provided a first look of the potential geographic extent of suitable area for dryland farming using rock piles in the borderlands of Sonora and Arizona. Likewise, using current climate data, MaxEnt created models for *A. angustifolia* cultivation in cattle-grazed areas across Sonora. These models highlighted potential areas for *Agave* cultivation along the edges of the designation-of-origin region for mescal bacanora (DOF, 2000). In addition, MaxEnt models highlighted suitable areas in the borderlands of Sonora and Arizona of two *Agave* species (*A. parryi* and *A. palmeri*) with potential to be used in the *Agave* agricultural industry (Mielenz *et al.,* 2015). *Agave* dryland-farming models, which incorporate data related to rock piles and cattle-grazed fields, provide an overview of the potential areas to cultivate *Agave* in lands with limited access to irrigation water.

Although rock piles are a pre-Columbian technology not currently used in the borderlands, they can be used to cultivate *Agave* and harvest rainfall water in the current climate. More recently, mescal farmers in Puebla, Mexico, where rain is scarce, harvest *Agave marmorata* in soils with high superficial stone content (Valenzuela-Zapata, 2020b). Farmers in the region have observed that *Agave* grown in rocks increases mescal palatability. In regions with 200 mm or less of annual rainfall, such as in semi-arid areas of Guanajuato, Mexico and South Africa, mescal farmers successfully cultivate *Agave salmiana* and *Agave americana* (Valenzuela-Zapata, 2019, 2020a). Since *Agave* dryland-farming practices generally do not use tillage, these techniques can enhance soil properties by increasing storage of rainwater (Hansen *et al.,* 2017). Our study also highlighted the potential of diversifying use of the land by using dryland farming and endemic *Agave* species in the *Agave* agricultural industry. Dryland farming and use of endemic *Agave* species, such as *A. palmeri* and *A. parryi*, can provide options that complement the bacanora industry in Sonora (Nuñez-Noriega, 2004).

Our models of dryland-farming systems and *Agave* species yielded values above AUC values of 0.90, suggesting that MaxEnt performed well in constructing prediction models for rock piles and grasslands suitable for *Agave* cultivation across Arizona and Sonora (Li *et al.*, 2016). In our study, MaxEnt calculated relatively high AUC model values (≥0.90), which suggests that the distributional extent of *Agave* dryland-farming can be forecasted, even with limited samples (Proosdij *et al.*, 2016). The AUC is a model performance measurement and validation tool generated by the MaxEnt algorithm (Hoffman *et al.*, 2008; Ardestani *et al.*, 2015; Phillips *et al.*, 2017; Elith *et al.*, 2019). Ardestani *et al.* (2015) suggested that AUC values from MaxEnt models with values greater than 0.90 are optimal, with values ranging between 0.80-0.90 and 0.70-0.80 considered to be moderate and acceptable. Area-under-the-curve values of dryland farming and *Agave* species were estimated to be <0.95 (Table 3). These values illustrate the power of MaxEnt to create models of the potential extent of rock piles and grasslands for *Agave* cultivation in the borderlands of Arizona and Sonora (Li *et al.*, 2016) (Figures 2-2 and 2-3). Additionally, our models provide information of suitable environmental and climatic conditions for dryland farming of *Agave*, indicating the optimal temperature and elevation range for its implementation to cultivate agaves in the region (Figure 2-4).

We assessed the accuracy of MaxEnt in creating suitability models for dryland farming and *Agave* species using a binomial-probability test (Table 2-3) (Pearson *et al.*, 2007; Phillips *et al.*, 2017). This nonparametric analysis has been suggested when using small sample sizes to test whether predictability of a suitability model is proportional to the samples used to create the model (Pearson *et al.*, 2007). The predictability analysis for rock piles using the binomial-probability test was marginally significant, yielding a *p*-value of 0.08, indicating that there were a sufficient number of rock-pile fields included in the analysis to construct a reliable rock-pile model to determine the potential extent of this dryland-farming system in Arizona and adjacent areas in Sonora (Figure 2-2a). Similarly, the predictability of the model for *Agave* grasslands yielded a statistically significant *p*-value of 0.03, showing that MaxEnt created a reliable model of the potential distributional extent of dryland farming for cultivating *A. angustifolia* in Sonora. The predictability of the models for *A. parryi* and *A. palmeri* yielded *p*-values of > 0.1, which were not statistically significant, thus indicating low predictability of MaxEnt for these two species. Overall, however, model results indicate fairly accurate predictability to identify areas for rock piles and *Agave* grasslands in the region (Figure 2-2a–c).

Distribution and suitable areas for Agave dryland farming

Our models predicted the distribution of rock piles throughout the Sonoran Desert region and grasslands across the Sierra Madre in Sonora (Figures 2-2a and b). Rock piles were predicted in the Sonoran Desert mainland, predominantly in southern and central Arizona (Figure 2-2b). The predicted distribution for rock piles included areas in the southeastern portion of Arizona in the borderlands with northwest Sonora, particularly near the municipalities of Caborca and Altar (Figure 2-2a). *Agave*-grassland farming was predicted throughout most of the state of Sonora, suggesting that *Agave* cultivated in grasslands are likely to succeed in more diverse environments and climates outside of the Sonoran Desert (Gentry, 1972; Cervantes Mendívil *et al.*, 2007; Nunez-Noriega *et al.*, 2008).

Our models highlighted two particular regions within Sonora with suitable climates to cultivate *A. angustifolia* using dryland farming: 1) the original designation-of-origin region for mescal bacanora (DOF, 2000; Valenzuela-Zapata, 2009); and 2) the Fuerte-Mayo region. The original designation-of-origin region for mescal bacanora (DOF, 2000; Cervantes-Mendívil *et al.,* 2007) includes 35 municipalities in the Sierra Madre (Appendix 2). However, in southern Sonora, the region of Fuerte-Mayo, which includes the municipalities of Huatabampo, Navojoa and Alamos, is currently outside of the boundaries of the designation-of-origin region for mescal bacanora (Figure 2-2c and 2-3c). The Fuerte-Mayo region was originally described by Gentry (1972) to be where there are several taxa within the *A. angustifolia* species complex. Given that mescal bacanora is produced traditionally and crafted in the mountain ranges in the Sierra Madre Occidental in Sonora, this region was not considered part of the designation-of-origin region for bacanora (DOF, 2000; Valenzuela-Zapata, 2009). Also, *A. angustifolia* is not commercially cultivated in the region (Burwell, 1995; Cervantes- Mendívil *et al.*, 2007). The Mexican designations of origin differ from those used in Europem, in that in small geographic regions and traditional products are named after towns, such as for mescal bacanora that is suspected that originated in the town of Bacanora in northern Sonora (Valenzuela-Zapata & Macías-Macías, 2014). However, conflict could arise if the designation-of-origin region for bacanora is expanded, potentially creating rivalries within the *Agave* agricultural industry in Sonora (Salazar-Solano, 2007; Salazar-Solano & Mungaray-Lagarda, 2009). Given the diversity of *Agave* species in Sonora, the mescal bacanora industry can be complemented with other traditional spirit drinks produced artisanally across Sonora (Castetter *et al.,* 1938; Gentry, 1972). Besides bacanora, different mescals have historically been produced in Sonora along the edges of the designation-of-origin region for bacanora (Salazar-Solano, 2007).

Suitable environments and climate for rock piles vs. Agave grasslands

Our models indicated environmental differences in predicted distributions of rock piles and *Agave*-grassland areas within the region (Figure 2-2a and c). Apparent lower precipitation in predicted suitable areas for rock piles relative to predicted suitable areas for grasslands were extracted from the models (Figure 2-4b and c). For south-central Arizona and the southeastern portion of the borderlands with Sonora, precipitation is lower than along the Sierra Madre and southern Sonora where *Agave* grasslands were predicted. Rock piles and grasslands also differed in elevation (Figure 2-4a). Although predicted rock piles and grassland areas had similar elevations between 500-1000 m above sea level, suitability for rock piles decreased at 1500 m above sea level (Figure 2-4a). Our models indicated that grasslands occupy a range of elevations in Sonora between 500-2000 m in the Sierra Madre Mountains (Cervantes-Mendívil *et al.*, 2007; Núñez-Noriega *et al.*, 2008). However, areas for rock piles are more likely to be found in mesic environments of the Sonoran Desert in south-central Arizona, which are generally found in lower elevations, which is consistent with rock-pile fields in central Arizona located between 600-900 m (Fish & Fish, 1992).

Hill slope and air temperature were factored into past models of productivity of agaves in the region (Nobel & Hartsock, 1986; Garcia-Moya *et al.*, 2011; Lewis *et al.*, 2015). Nobel and

Hartsock (1986) reported that productivity of *Agave deserti* was correlated with slope-face direction. Cervantes-Mendívil *et al.* (2007) observed in grazing areas, which were cultivated with *A. angustifolia,* that plants adapted better and survived more often in sites with low temperatures above 1°C than in sites with temperatures between -2 to -8 °C in the Sierra Madre in Sonora. Nuñez-Noriega *et al.* (2008) suggested that in cattle-grazing areas, temperatures at elevations between 800-1200 m are ideal for *A. angustifolia* cultivation. Fish and Fish (1990, 1992) suggested that in rock-pile fields in mesic environments with elevations between 600-900 m, *Agave* species are less susceptible to low temperatures. Our models suggest that while slope and temperature are important environmental factors for site selection for cultivation and for plant survival, they carry less influence than precipitation and elevation in predicting the potential distributional extent of dryland-farming within the region (Table 2-3 and Figure 2-4).

While previous models assessed suitable cultivation areas for *Agave* using a theoretical multi-criteria approach in the region (e.g., climate and productivity indexes and GIS) (Lewis *et al.*, 2015), such models did not include dryland farming as a factor to forecast *Agave*-cultivation areas. However, the extent of dryland farming predicted in our models indicated similar areas for *Agave* cultivation in Arizona as the models reported by Lewis et al. (2015). These results suggest southern and central Arizona are regions with high potential for dryland farming. Additionally, our models indicated suitable environments and climate with high potential for application of *Agave* dryland farming, particularly using rock piles in the borderlands of Arizona and Sonora (Figure 2-4).

Differences between dryland-farming-system and Agave-species models

For *Agave*-grassland farming in Sonora, permutation analysis indicated overlap with the native range of *A. angustifolia*, suggesting that the environments for cultivation in grasslands are similar to environments where *A. angustifolia* naturally occurs. Conversely, the analysis of our models showed little or no correlation between suitable environments for rock piles and *A. palmeri* and *A. parryi*, which occur in the borderland region of Arizona and Sonora (Table 2-4). Comparisons of rock piles with these two *Agave* species suggest that their predicted ranges likely occur outside of the predicted areas for rock piles. In addition, the predicted suitable climate for these two species is likely found outside existing rock-pile fields in south-central Arizona.

Two *Agave* species, *Agave murpheyi* and *Agave sanpedroensis*, have been reported to have been cultivated by the Hohokam using rock piles (Fish & Fish, 1992; Adams & Adams, 1998; Hodgson *et al.*, 2019). Similarly, *A. marmorata*, which is harvested from the wild in Puebla, Mexico, has been observed to grow well in areas with high rock content (Valenzuela-Zapata, 2020b). Evidence of historic uses of *A. palmeri* and *A. parryi* has been found at archaeological sites (Appendix 2-3) (Minnis & Plog, 1976; Nabhan *et al.*, 2019). However, these two *Agave* species have been found outside the native distributions of *Agave* species that were cultivated using Hohokam rock piles (Hodgson *et al.*, 2019).

Based on the analysis of suitability models for rock-pile fields and agaves (Figure 2-4), our results indicate that *A. palmeri* and *A. parryi* require more moisture than where rock-pile fields are generally located (Pavliscak & Fehmi, 2020). Likewise, our results (Figure 2-4b) suggest that these two species occur in more moist environments than Hohokam rock-pile fields sites in south-central Arizona region as reported by Fish & Fish (2014). Wild species of *A. palmeri* and *A. parryi* have been reported in similar environments within the borderlands of Arizona and Sonora (Fish & Fish, 2014; Hodgson *et al.*, 2019; Nabhan *et al.*, 2019). In addition, these two endemic species are conventionally used to produce mescal in the borderlands (Klopper *et al.*, 2010), and research on applications of dryland farming to cultivate these two species is needed. Similarly, more research is needed to characterize historical cultivation of these two *Agave* species (Appendix 2-3) (Nabhan *et al.*, 2019). Knowledge about the suitability and similarities of dryland farming and *Agave* species, based on their climatic requirements and distributions, can be used as a tool to identify more *Agave* species that potentially could be cultivated using dryland farming in the future. Although using permutation tests allowed us to analyze overlapping between dryland farming areas and the native ranges of *Agave* species, accuracy of this method might be affected by sample sizes (Proosdij *et al.*, 2016), which requires further experimentation.

Comparison of Agave angustifolia to Agave palmeri and Agave parryi

Agave angustifolia is the most abundant *Agave* species in Sonora (Gentry, 1972; Cervantes-Mendívil *et al.*, 2007; Núñez-Noriega *et al.*, 2008). Forecasting the suitable distribution of *A. angustifolia* and the possible overlap with the native ranges of other *Agave* species, such as *A. palmeri* and *A. parryi,* provides a clearer sense of geographic boundaries between these species within the region and with dryland farming in the borderlands of Arizona and Sonora. Upper distributional limits of *A. angustifolia* were predicted in the north-central part of Sonora, adjacent to where *A. palmeri* and *A. parryi* naturally occur (Figure 3 a, b, c).

Comparisons of Agave palmeri vs. Agave parryi models

Despite the fact that the predicted suitable models showed similarities between wild *A. palmeri* and *A. parryi* species (Figure 3 a and b), the permutation test indicated that distributions of these two species are different in their predicted environments within the region. Our results comparing suitable distribution models for these two *Agave* species using the permutation test showed that when extracting permutation scores from these models using ENMTools, the observed value (0.42) was lower than the critical value (0.94), indicating differences in their predicted distributions. Field work is needed to estimate the extent of the distributional range of these two *Agave* species. Field observations by Nabhan *et al.* (2019) suggests that some wild *A. parryi* species, such as the subspecies *A. parryi* var. *huachucensis*, overlap with wild *A. palmeri* species.

Future research to forecast dryland-farming and Agave cultivation

The economic potential of *Agave* as a drought-tolerant crop for arid regions has been extensively characterized (Davis *et al.,* 2011, 2017, 2019; Garcia-Moya *et al.,* 2011; Stewart, 2015). However, despite the substantial research on the impacts of climate change on *Agave*, little research has been conducted on *Agave* dryland-farming cultivation in the borderlands of Arizona and Sonora (Ortiz-Cano *et al.,* 2020). Current patterns of climate changes pose challenges to the *Agave* agricultural industry in Sonora. In order to continue producing mescal bacanora, research is needed to find sustainable methods to produce *A. angustifolia* (Nuñez-Noriega, 2004; Nuñez-Noriega *et al.,* 2008). Finding endemic *Agave* species that complement the current *Agave* industry in Sonora is also crucial. By diversifying *Agave* cultivation in Sonora and the borderlands with Arizona, producers can expand beyond production of mescal to develop other products derived from *Agave*, such as feedstock, syrups, biofuel, synthetic drugs, fructans, and saponins (Cushman *et al.,* 2015; Stewart, 2015; Davis *et al.,* 2019). An increase in the cultivation of *Agave* in the borders could also boost the economy of the region (Davis *et al.,* 2019). The *Agave* industry in Sonora is gaining a stronger economic foothold in the EU and U.S. markets (Stewart, 2015; DOF, 2020). Moreover, agronomical research for exploring *Agave* as crop in the borderlands needs a collaborative effort between Mexico and the U.S. in the context of current climate change (Nuñez-Noriega, 2004).

This is the first time MaxEnt modeling has been used to predict potential areas suitable for *Agave* dryland farming in Arizona and Sonora. MaxEnt has primarily been used in ecological-niche studies to find species-specific locations (Phillips *et al.*, 2017). The purpose of our study was to use MaxEnt to identify areas for potential *Agave* cultivation. A similar approach was reported by Healy *et al.* (2017) to assess ancient dryland farming in New Mexico. Although MaxEnt can estimate suitability-distribution models with limited samples (Proosdij *et al.*, 2016), we found our results to be constrained because the exact geographic boundaries of our models cannot be confirmed without extensive field work. More specifically, two factors likely affected our results: 1) rock-pile fields were less abundant in the region relative to the abundance of grassland fields, and 2) limited information is available regarding the two dryland-farming systems in the borderlands. Only a small number of rock-pile fields and *A. angustifolia*-grasslands fields have been agronomically studied in Arizona and Sonora (McDaniel, 1985; Cervantes-Mendívil *et al.*, 2007; Núñez-Noriega *et al.*, 2008). Depending on sample size and geographic extent selected to produce suitability models, two kinds of models can be predicted using MaxEnt: widespread models (i.e., large sample size) and narrow-range models (i.e., minimal to medium sample size) (Proosdij *et al.*, 2016). Based on the limited availability of samples for this study, our models fit in the category of narrow-range models. As such, interpretation of our results should be treated with caution. Future studies likely need a more refined search of dryland farming in the region to better address the ratio of predicted and known samples in the borderlands region. In addition, experiments with reciprocal common gardens with rock piles cultivated with agaves would enhance our understanding of the potential application of rock piles in *Agave* production.

CONCLUSIONS

The ecological-niche-modeling software, MaxEnt, identified potential areas for *Agave* cultivation in the borderlands of Arizona with Sonora. Although there are constraints in our study, MaxEnt prediction maps and environmental variable analyses of *Agave* dryland farming and *Agave* species models provide a strong foundation for future research on *Agave* cultivation in the region.

The use of rock piles, which have similar properties to mulch to retain moisture and buffer soils from high temperatures, is a promising water-conserving method to cultivate *Agave* in the Sonoran Desert. Our research determined potential areas for *Agave* rock-pile cultivation beyond the known archaeological range in the Tucson-Phoenix Basin in the southwestern portion of Arizona in the borderlands with Sonora. Potential areas with suitable climates for *Agave* cultivated using grasslands were mainly found outside the Sonoran Desert region, but throughout other parts of the state of Sonora, including two regions: 1) the designation-of-origin region for bacanora (Appendix 1) and 2) the Fuerte-Mayo region (Figure 2c and 3c). Our models identified southern Sonora, primarily the Fuerte-Mayo region, as a potential area in the outer limits of the range of the designation-of-origin region to cultivate *A. angustifolia* for bacanora production, particularly in grasslands (Cervantes-Mendívil *et al.*, 2007). Additionally, our models highlighted southern Arizona and the borderlands of north-central Sonora as a suitable region for cultivating *A. palmeri* and *A. parryi* (Figure 2a and b). Our models suggest that *A. angustifolia* might be found in the same areas identified for potential dryland farming of agaves in grasslands (Figure 3a and 3b). Based on the predicted distribution models of rock piles and grasslands, our models suggest that dryland farming has potential to be implemented in Sonora and Arizona to cultivate agaves. Future agricultural and agroecological studies on rock piles and *Agave* grasslands are necessary to understand their application in these areas.

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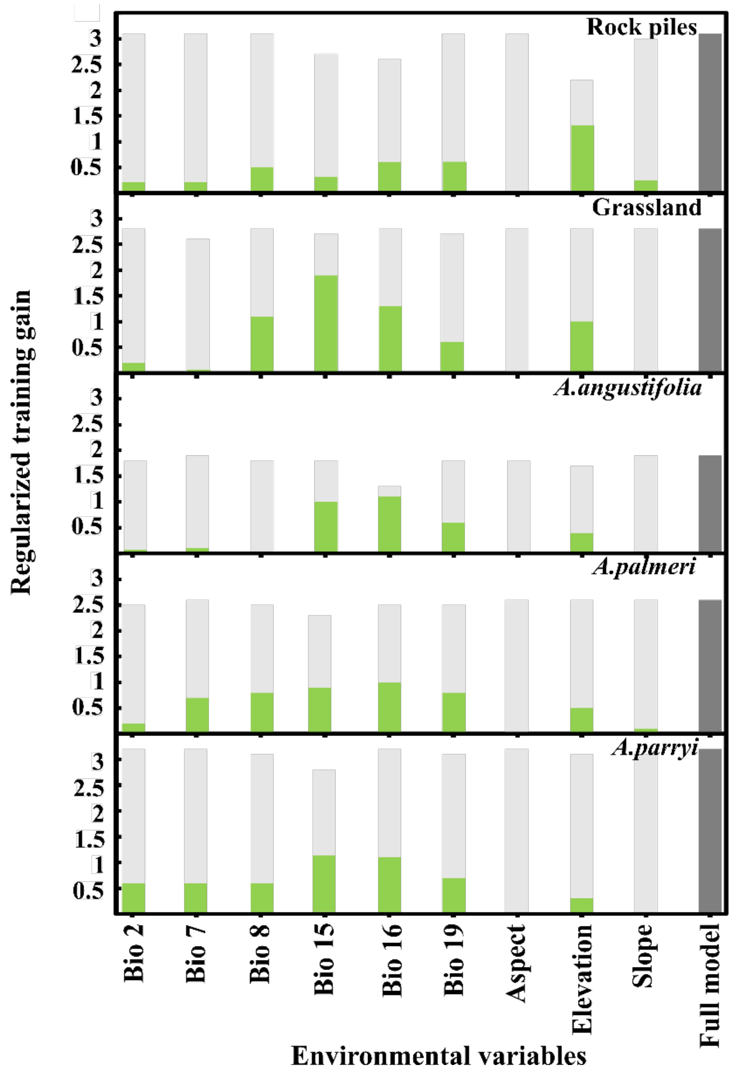


Figure 2-1. Summary of the importance of environmental variables in the development of suitability models for Agave dryland-farming (a, b) and Agave species (c, d, e) relative to regularized training gain. Green bars indicate model gain when only including individual environmental variables. Light-grey bars show the gain when the individual environmental variables are excluded from the full model. Dark-grey bars indicate the gain achieved in the full model, including all environmental variables in the model. Abbreviated environmental variables are defined as follows: Bio 2 = mean diurnal range (mean of monthly (maximum temperature - minimum temperature)), Bio 7 = temperature annual range (maximum temperature – minimum temperature), Bio 8 = mean temperature of wettest quarter, Bio 15 = precipitation seasonality, Bio 16 = precipitation of wettest quarter, and Bio 19 = precipitation of coldest quarter.

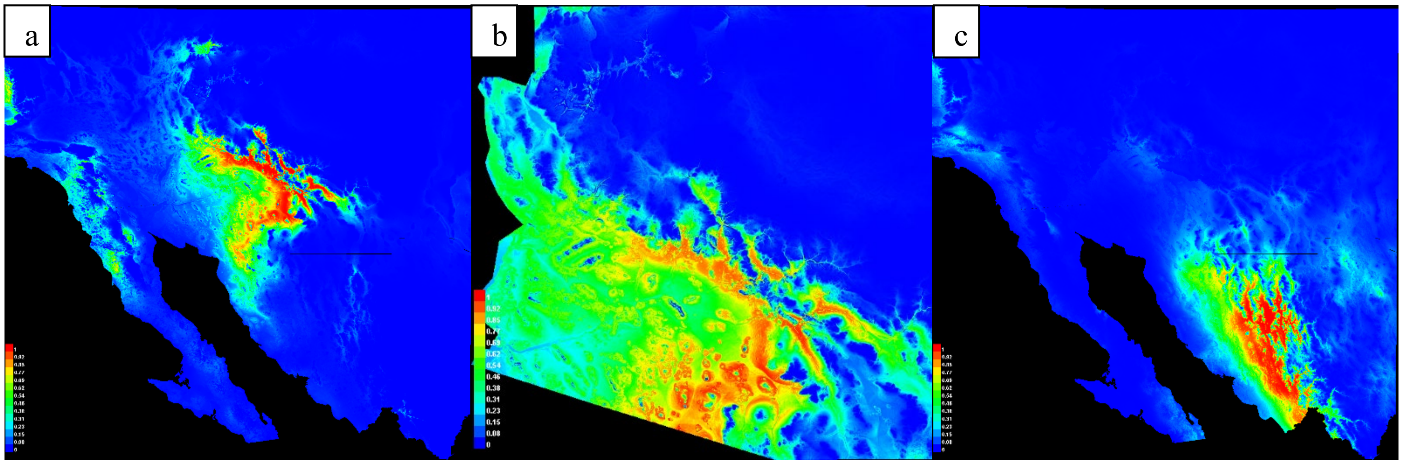


Figure 2-2. Suitability models for rock piles in the Sonoran Desert region (a), Arizona, USA (b), and for Agave grasslands in Sonora, Mexico (c). Coloration in red indicates high suitability potential for rock piles in (a) and (b). Coloration in red in (c) indicates high suitability for grasslands.

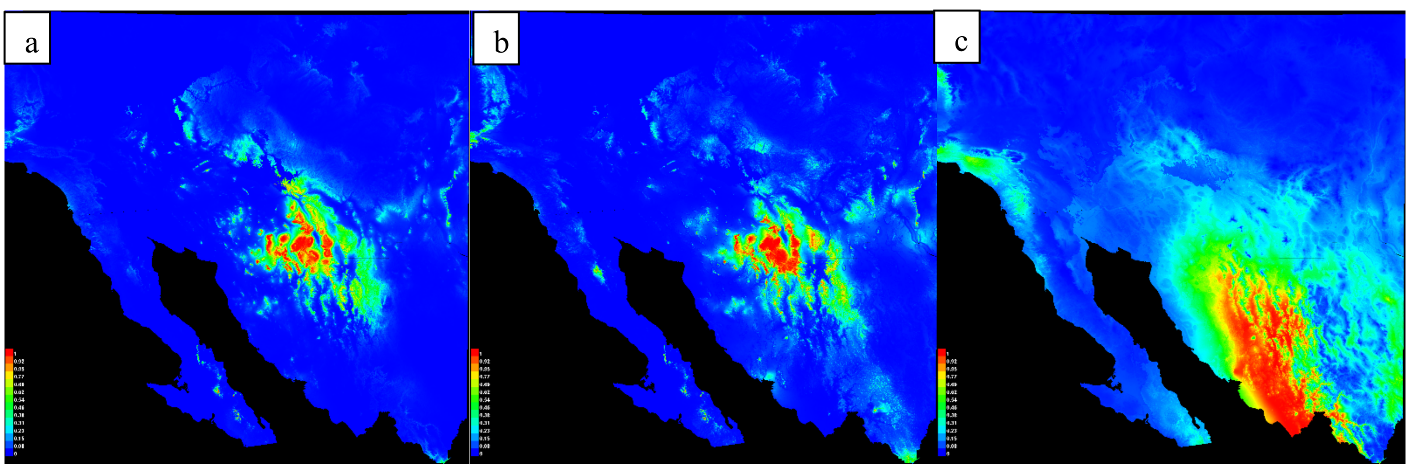


Figure 2-3. Suitability models of *Agave palmeri* (a), *Agave parryi* (b), and *Agave angustifolia* (c) in the borderlands of Arizona, USA and Sonora, Mexico. Red color indicates high suitability for *Agave* species.

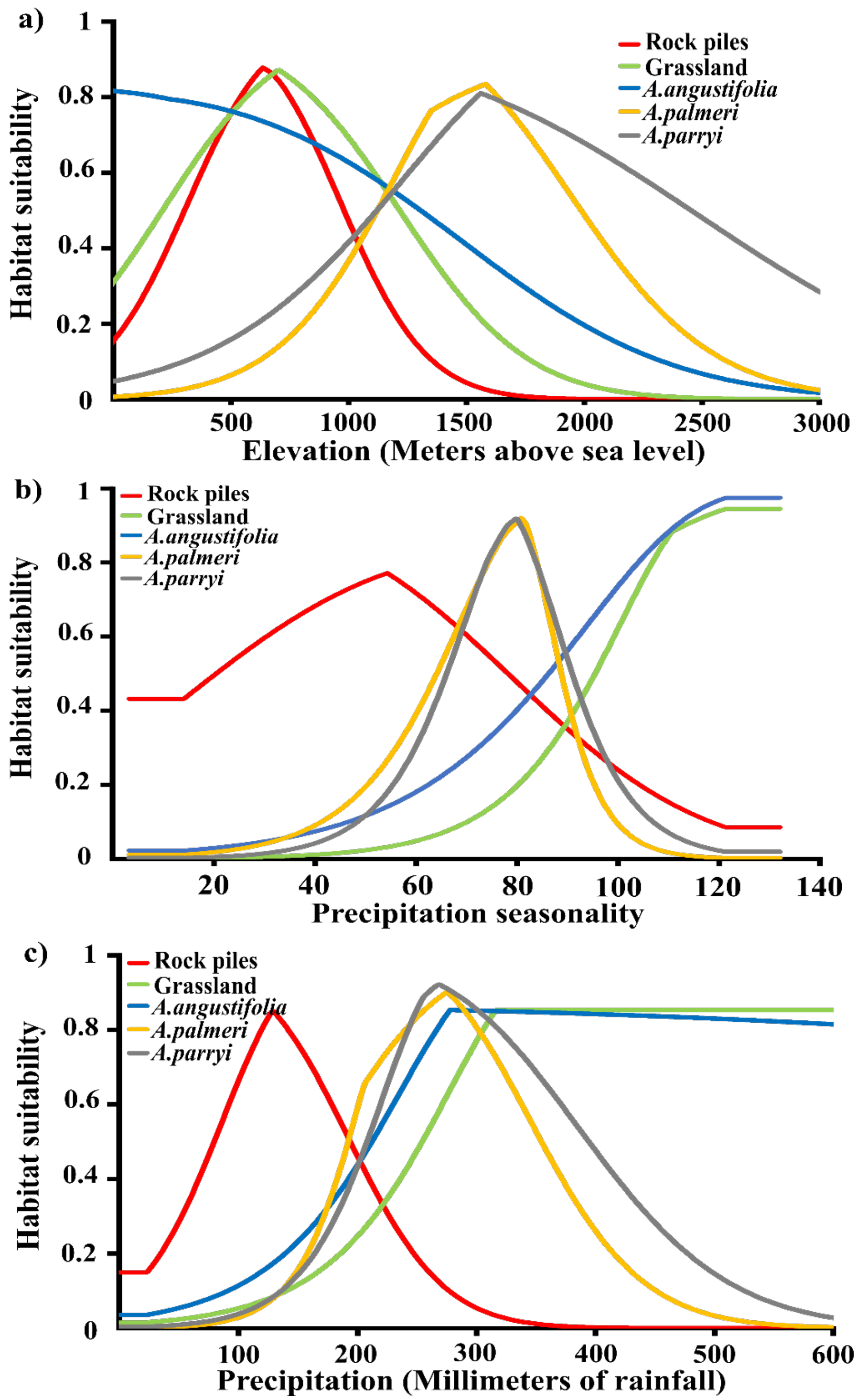


Figure 2-4. Habitat suitability for rock piles, grasslands, and *Agave* species (*A. angustifolia*, *A. palmeri*, and *A. parryi)* during the wettest season in a given year in terms of three environmental variables: (a) elevation, (b) precipitation seasonality, and (c) annual precipitation.

TABLES

Table 2-1. Sites and numbers of samples evaluated in our study of rock piles (top portion of table) and grasslands cultivated with Agave angustifolia (bottom portion of table) using MaxEnt.

Archaeological sites with rock piles in Arizona, USA	**Samples per site**
Tumamoc Hill, southern AZ	12
Marana, southern AZ	10
Santan Mountains, central AZ	4
Horseshoe Lake, central AZ	18
Grasslands cultivated with *Agave* in Sonora, Mexico	**Samples per site**
Mátape, central SON	1
San Pedro de La Cueva, central SON	1
Bacanora, central SON	1
Pueblo de Álamos, Ures, central SON	1
Álamos, southern SON	1
Tepache, northern SON	1
Villa Hidalgo, northern SON	2
Arizpe, northern SON	1
Banamachi, northern SON	1
Moctezuma, northern SON	5

Table 2-2. Environmental variables derived from monthly temperature and rainfall values. The variables were selected from the WorldClim database to generate dryland-farming MaxEnt models.

Environmental predictor variables:
Bio 2= mean diurnal range (mean of monthly (maximum temperature - minimum temperature))
Bio 7= temperature annual range (maximum temperature - minimum temperature)
Bio 8= mean temperature of wettest quarter
Bio 15= precipitation seasonality (coefficient of variation)
Bio 16= precipitation of wettest quarter
Bio 19= precipitation of coldest quarter
Aspect
Elevation
Slope

Table 2-3. Summary of permutation importance, via MaxEnt analysis, for each individual variable in the dryland-farming and Agave-species models.

	Environmental permutation importance (%)								
Species	**Slope**	**Aspect**	**Elevation**	**Bio 2**	**Bio7**	**Bio 8**	**Bio 15**	**Bio 16**	**Bio 19**
Rock piles	1	0	64.6	0	0	0.2	19.7	14.3	0.2
Grassland	0	0.3	10	0	4.8	3.1	72.6	0.1	9.1
Agave angustifolia	1.2	0.1	7.2	0	0	7.7	0	83.8	0
Agave palmeri	0.4	0	8.1	12.7	0	43.6	16.3	5	13.9
Agave parryi	1.5	0	30.7	8.1	0	15.5	31.5	1	11.6

Table 2-4. Summary of niche models of individual species for dryland-farming and *Agave* species with test gains of all the variables used to build the distribution models with only one variable. The importance and contribution to the full model can be extracted from the estimated gain of each variable relative to the full model. Abbreviated environmental variables are defined as follows: Bio 2 = mean diurnal range (mean of monthly (maximum temperature - minimum temperature)), Bio 7 = temperature annual range (maximum temperature – minimum temperature), Bio 8 = mean temperature of wettest quarter, Bio 15 = precipitation seasonality, Bio 16 = precipitation of wettest quarter, Bio 19 = precipitation of coldest quarter, and Bio 19 = precipitation of coldest quarter.

Dryland-farming and *Agave* species	**Model test gain**		**Model accuracy**	**Test gain for individual variables**								
	Test AUC	**Full model**	***p*-values**	**Slope**	**Aspect**	**Elevation**	**Bio 2**	**Bio7**	**Bio 8**	**Bio 15**	**Bio 16**	**Bio 19**
Rock piles	0.99	3.14	0.08	0.15	0.63	0.15	0.59	0.62	0.43	0.43	0.17	0.28
Grassland	0.98	2.85	0.03	0.60	0.61	0.21	0.47	0.61	1.94E-01	0.29	0.56	0.31
Agave angustifolia	0.96	1.88	0.1	0.56	0.62	0.33	0.54	0.65	0.29	0.39	0.58	0.64
Agave palmeri	0.96	2.59	0.9	0.73	0.58	0.19	0.46	0.18	0.12	0.22	0.12	0.20
Agave parryi	0.98	3.41	0.3	0.97	0.70	0.32	0.63	0.38	1.82E-01	022	0.26	0.28

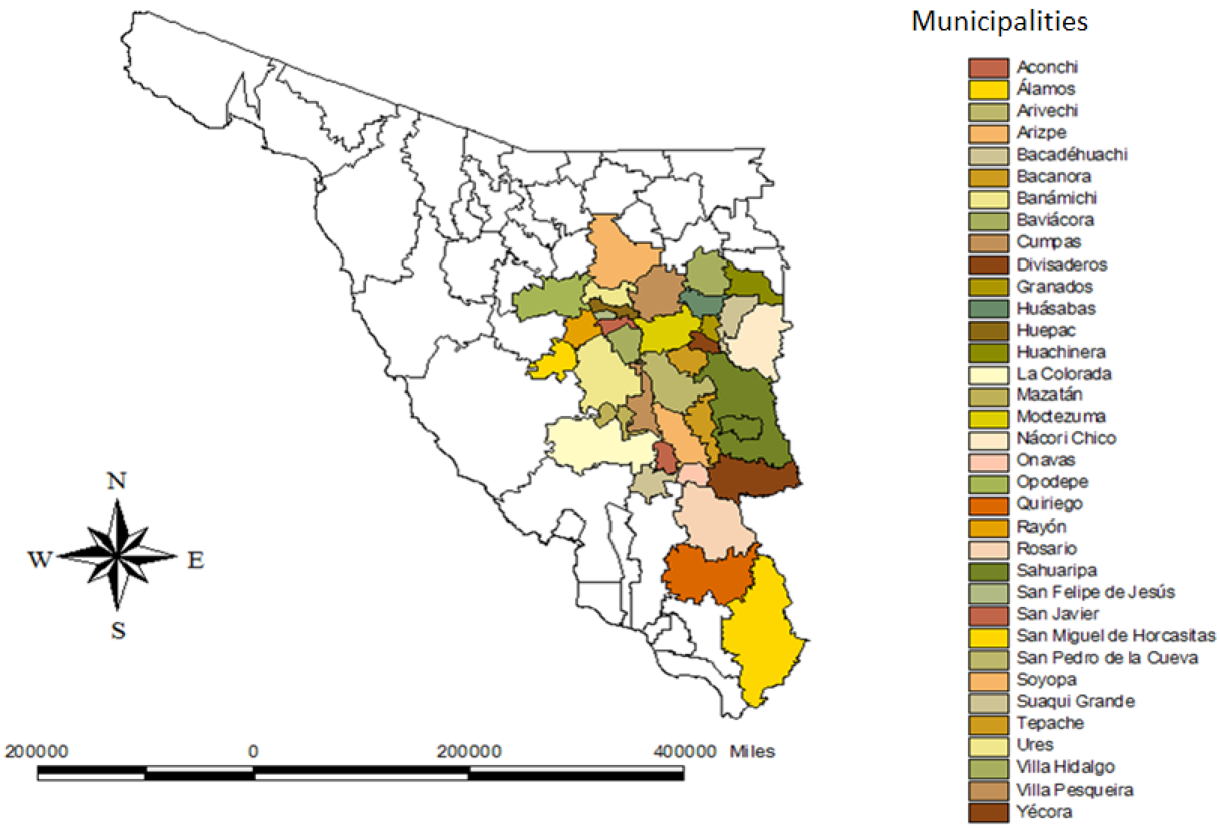
Table 2-5. Suitability and overlapping statistical assessment using permutation tests. Summary of the observed *I* values and critical *I* values calculated using the permutation tests. Significant differences between predicted suitable areas occur when observed *I* values are lower than the 5% critical *I* values, indicating non-identical suitable areas for dryland-farming and *Agave* species. Bold values indicate significant differences between paired suitable areas calculated in the permutation test.

Suitable areas comparisons	**Observed *I***	**5% critical *I***
Rock piles vs. grassland	0.52	0.47
Rock piles vs. *Agave palmeri*	**0.39**	**0.46**
Rock piles vs. *Agave parryi*	**0.21**	**0.34**
Agave palmeri vs. *Agave parryi*	**0.42**	**0.92**
Agave palmeri vs. *Agave angustifolia*	**0.34**	**0.64**
Agave parryi vs. *Agave angustifolia*	**0.17**	**0.71**
Grassland vs. *Agave angustifolia*	0.45	0.43

APPENDICES

Appendix 2-1. Map of the borderland states of the U.S. and Mexico. The area highlighted in grey was the study area used to create suitability models for *Agave* dryland farming and *Agave* species.

Appendix 2-2. Designation of origin for mescal bacanora in Sonora, Mexico.



Appendix 2-3. *Agave parryi* plant growing in an ancient rock pile at an archaeological site in Casas Grandes, Chihuahua, Mexico (picture courtesy of M. Searcy, 2019).

CHAPTER 3

Rock Mulching Insulate Soils and Increases CO_2 Uptake of *Agave* Species

Hector Genaro Ortiz-Cano[1], Robert Hadfield[1], Taiyo Walters[1], Steven L. Petersen[1], Neil C. Hansen[1], Michael T. Searcy[2], Ricardo Mata-Gonzalez[3], Kevin Hultine[4], Antonio Villanueva-Morales[5], Pilman Park[6], J. Ryan Stewart[1]

[1]Brigham Young University, Department of Plant and Wildlife Sciences, Provo, Utah, USA
[2]Brigham Young University, Department of Anthropology, Provo, Utah, USA
[3]Oregon State University, Department of Animal and Rangeland Sciences, Corvallis Oregon, USA
[4]Desert Botanical Garden, Phoenix, Arizona, USA
[6]Universidad Autónoma Chapingo, División de Ciencias Forestales, Departamento de Estadística, Matemática y Cómputo, Chapingo, Mexico
[6]Floriculture Research Division, National Institute of Horticulture and Herbal Sciences, Rural Development Administration, Jeonju, South Korea

ABSTRACT

A pre-Columbian indigenous group, Hohokam, used rock piles as mulching to cultivate *Agave* in Arizona, USA. Rock piles acted as mulch to retain soil moisture and catch rainfall runoff from hill slopes. These rock piles appear to be their main approach for cultivating drought-tolerant *Agaves*. *Agave murpheyi* (Hohokam Agave) is among the *Agaves* in the region cultivated by the Hohokam. *Agave murpheyi* has been reported to be endemic at archaeological sites in Arizona. This species also was cultivated extensively using rock piles in Arizona. *Agave americana* has been reported as a species with high potential to be cultivated in the U.S. These two *Agave* species have the potential to produce large amounts of edible biomass under limited-water conditions in the current and future climates. However, little is known of modern dryland farming using rock mulching to cultivate *Agaves*. Few studies have addressed how insulative properties of rock piles likely contribute to bolster drought tolerance, CAM metabolism, and

productivity of *A. murpheyi*. Little is known about of the effect of using rock mulching to cultivate *A. americana* and how this cultivation method could affect the physiological performance of *A. americana*. We hypothesized that soil moisture and temperature in rock piles affect CAM efficiency by increasing the nocturnal CO_2 uptake of *A. murpheyi* and *A. americana*. In field- and lab-based experiments using 3-year-old *A. americana* and *A. murpheyi* plants growing in experimental rock piles, we compared nocturnal CO_2 uptake in bare soil and rock piles. Real-time nocturnal CO_2 measurements were made using a portable infrared photosynthesis and CO_2 gas analyzer in the summer months of 2018 and 2019. The equipment was adapted to sample night-time, leaf-level CO_2-gas exchange. While measuring CO_2 of *Agave*, we measured soil temperatures, moisture content, and water-potential values from rock piles and bare soil. We also evaluated the relationship of these variables with CO_2 uptake. We observed higher CO_2 uptake in rock piles than in bare soil. The differences found were associated with temperature and moisture underneath rock piles. Rock piles promoted comparatively higher soil moisture and cooler soil temperatures during the day and warmer temperatures below rocks at night than found in bare soil. Rock piles reduced soil evaporation and increased moisture content and water potential relative to bare soil. Daily temperatures and moisture below rocks were beneficial to the metabolic response of *Agaves* by reducing drought and heat stress. Although more research is needed for using rock piles to cultivate *Agave*, the insulative properties provided by rock piles offers an alternative to cultivate *Agave* in current and future climate change.

INTRODUCTION

The Hohokam were a pre-Columbian, indigenous group, which extensively practiced irrigation agriculture techniques in the Sonoran Desert (Fish et al., 1985). In addition, they used dry farming and rainwater harvesting techniques to cultivate the desert (Fish and Fish, 1990; Masse, 1979). They cultivated *Agaves* in Arizona using rock mulching, also known as Hohokam rock piles (Fish and Fish, 1990, 1992; Homburg and Sandor, 2011; Lightfoot, 1996; Masse, 1979). Hohokam rock piles, which are the main rainwater harvesting technique to cultivate *Agave* found at archaeological sites in the Sonoran Desert, vary in shape and size. Masse (1979) found rock piles with mound shapes averaging 0.5 m in height and 5–10 m in diameter at an archaeological site at Tumamoc Hill in Tucson, Arizona. Fish et al. (1985) found rock piles with heights from less than 0.75 m to nearly 1.5 m at a Hohokam archaeological site in the Tortolita Mountains in Arizona. Hohokam rock piles are an artificial modification of soil microtopography (Lightfoot, 1996, 1994; Wilken, 1972). The main function of rock piles appears to have been to retain soil moisture and to channel rainfall underneath rocks (Brevik et al., 2016; Homburg and Sandor, 2011).

Rock piles are micro-topographic modifications to soil designed to catch rainwater (Sandor and Homburg, 2011; Wilken, 1972). The Hohokam constructed rock piles at toe slopes of mountains and upland hillslopes (Fish and Fish, 1990, 1992; Homburg and Sandor, 2011). Mountain slopes maximized rainwater interception in rock piles, which allowed for rainwater infiltration below the rocks (Homburg and Sandor, 2011; Sandor and Homburg, 2011). Organic matter and nutrients mixed in the runoff were deposited underneath rocks, which enriched the rhizosphere of *Agaves* growing in the rock piles (Brevik et al., 2016; Sandor and Homburg, 2011). A less explored aspect of rock piles is their ability to insulate soils from high temperatures (Wilken, 1972); research has not been done yet to evaluate the thermal properties of pre-Columbian rock mulching. Understanding insulation properties of rock piles is important for future *Agave* cultivation, knowing that soil temperatures in the Sonoran Desert reach up to 70–80 ˚C (Cui and Nobel, 1992; Franco and Nobel, 1989; Nobel, 2003) and in the future soil temperatures will be higher as temperature continue rising in the region (Aparecido et al., 2020).

Among the physiological features of *Agave*, CAM photosynthesis acts as the principal trait contributing to its productivity in hot and dry climates (Borland et al., 2009; Lüttge, 2004; Stewart, 2015). Crassulacean acid metabolism enables *Agaves* to close stomata during the day and open during the night to efficiently uptake CO_2 (Borland et al., 2011; Holtum and Winter, 2014; Nobel and Hartsock, 1983). This mechanism significantly reduces water loss from *Agaves* (North and Nobel, 1991). In dry regions, evapotranspiration losses of C_3 and C_4 crops tend to be high due to hot daytime temperatures (Nobel, 1991). Such crops need large amounts of irrigation water to compensate for water losses (Pimienta-Barrios et al., 2001). Using dryland-farming techniques such as rock mulching to cultivate *Agaves* can reduce irrigation water use in increasingly dry and hot regions (Ortiz-Cano et al., 2020; Stewart, 2015).

Morphological and physiological traits of *Agaves* make them resilient to drought (Garcia-Moya et al., 2011a; Nobel, 2003; Thiede, 2020). Traits such as their rosette shape and shallow root systems allow *Agaves* to assimilate relatively small amounts of atmospheric water in the form of rain, dew, or fog (De Micco and Aronne, 2012; Hodgson, 2001; Martorell and Ezcurra, 2007; Thiede, 2020). Counterintuitively, shallow root systems enable Agaves to absorb small amounts of rainwater (Martorell and Ezcurra, 2007; Nobel et al., 1989; North and Nobel, 1991; Palta and Nobel, 1989, 1989). In addition, the rosette-shaped canopy of *Agaves* funnel rainwater and dew droplets to their root systems (Martorell and Ezcurra, 2007; Thiede, 2020). Succulent leaves of *Agaves* store water and allow them to survive even in rain-limited years (Andrew et al., 1986; Linton and Nobel, 2001; Nobel, 2003).

In the present day, the U.S Southwest has a semi-arid climate suitable to cultivate *Agave* crops on an industrial scale (Davis et al., 2019, 2011). Through the use of a GIS land multicriteria analysis to assess climate and regions across the U.S. for *Agave deserti* and *Agave tequilana* cultivation, Lewis *et al.* (2015) found that the climate, land, and infrastructure in Arizona can support establishment of *Agave* plantations in the region. They suggested that this is possible due to the ability of *Agave* to adapt to harsh climates where conventional crops struggle to sustain yields, such as in Arizona (Davis et al., 2011; Lewis et al., 2015; Pérez-Pimienta et al., 2017).

While the drought tolerance of some species in the *Agave* genus, such as *Agave americana*, is well characterized (Abraham et al., 2016; Borland et al., 2009; Davis et al., 2017; Ehrler, 1983; Escamilla-Treviño, 2012; Garcia-Moya et al., 2011a; Neales, 1970; Nobel, 2003), the potential of other species, such as pre-Columbian cultivated *Agaves Agave murpheyi* and *Agave sanpedroensis*, remains unknown (Adams and Adams, 1998; Hodgson et al., 2018; Hodgson and Salywon, 2013; Parker et al., 2014). In recent years, new evidence has emerged of cultivated *Agaves* associated with Hohokam pre-Columbian cultivation using rock piles in the borderlands of northwest Mexico and Arizona (Fish and Fish, 2014; Hodgson et al., 2018). The species *Agave murpheyi* and *Agave sanpedroensis* can be found growing naturally in south-central Arizona (Hodgson et al., 2018; Kamermans et al., 2014). A unique aspect of these two species is that they have been found only at Hohokam rock piles (Fish and Fish, 2014; Hodgson et al., 2018). Putative *Agave* cultigens in the borderlands from pre-Columbian times include *Agave verdensis, Agave phillipsiana,* and *Agave delamateri* (Hodgson, 2001; Hodgson et al., 2018; Hodgson and Salywon, 2013; Parker et al., 2007).

In a dry region like the Sonoran Desert, understanding of the drought tolerance of *Agaves* and dryland-farming techniques is essential to continue using this crop through future climate changes. Particularly, basic agricultural aspects of *Agave*, such as biomass productivity, drought tolerance, cold hardiness, and carbon assimilation provide insights into its potential adaptations to heat and drought by means of using dryland farming (Davis et al., 2017; Niechayev et al., 2019; Nobel, 2003; Thiede, 2020). The effect of rising temperatures on *Agave* has been studied by Winter and Holtum (2014), indicating that high temperatures at night limit *Agave* biomass productivity, which is of particular concern given rising temperatures and increasing drought events in arid regions (Stewart, 2015). Only a small number of studies have reported using rock piles and using rainwater to cultivate *Agave* in the region (Creswell and Martin, 1998; Fish and Fish, 2014; McDaniel, 1985).

Using dryland-farming cultivation methods is crucial to support the demand for *Agave* as a crop in the future (Burwell, 1995; Lewis et al., 2015) despite ongoing climate change in arid and semi-arid regions (Stewart, 2015). A pre-Columbian agricultural system, such as rock mulching, may be a suitable option to sustainably cultivating *Agave* as a crop in the U.S.-Mexico borderlands (Ortiz-Cano et al., 2020). Only a small number of studies have reported dryland-farming techniques using solely rainwater to cultivate *Agave* in arid regions (Creswell and Martin, 1998; Fish and Fish, 2014; McDaniel, 1985).

Studies of Homburg et al. (2011, 2015), Wilken. (1972), Lightfoot et al. (1994, 1996) indicated that insulation properties of rock mulch benefit soil moisture in arid regions. Similarly, in a field experiment cultivating *Agave* in rocky soils, Nobel et al. (1992) observed that rocks facilitated moisture infiltration, increased moisture content in the soil, and favored *Agave* biomass accumulation. Likewise, Cui and Nobel (1991) reported that rocks increased soil moisture and *Agave* carbon assimilation. However, for successful application of *Agave* dryland farming in marginal arid lands, it is necessary to perform studies that couple CAM photosynthesis, water-use efficiency, and carbon uptake of *Agave* using dryland-farming methods (Jaradat, 2010). Capitalizing on physiological and morphological traits of *Agave* are crucial for its successful cultivation in the current climate (Stewart, 2015; Thiede. 2020). Among *Agave* physiological traits, nocturnal carbon uptake is pivotal for biomass accumulation in arid regions (Garcia-Moya et al., 2011).

Rock mulching is a promising method to cultivate *Agave* with potential to be used in the current environmental conditions (Ortiz-Cano et al., 2020). However, field research to test insulation properties of rock piles has not been done yet, largely because rock-pile fields are remote and there is limited access to archaeological sites. Fish and Fish (2014) conducted the only experiment that measured productivity of *Agave* in rock piles at a Hohokam rock pile field archaeological site. Conducting research on rock piles and *Agave in situ* at archaeological sites is complex given state requirements and tribal land regulations as outlined in the Federal Antiquities Act (16 U.S.C. 431-433) (U.S. Department of the Interior, 2021).

We designed an experimental rock pile field to test thermal properties of rock mulching and evaluate photosynthesis of *Agave* under low soil-water availability conditions. The main purpose of our study was to model Hohokam rock piles to evaluate the insulation effect of rock mulching on *Agave* physiology. We also sought to evaluate nocturnal carbon uptake performance of *Agave* relying solely on rainwater. In addition, our experiment enabled us to test the capacity of rock piles to harvest rainwater. To evaluate CO_2 uptake of *Agave*, we used an endemic Sonoran Desert pre-Columbian species, *Agave murpheyi,* which was intensively used and cultivated by the Hohokam using rock piles (Adams and Adams, 1998; Fish and Fish, 2014), and *Agave americana,* which is cultivated in arid and semi-arid climates around the world (Thiede, 2020). We evaluated nocturnal CO_2 uptake patterns of these two *Agave* species in rock piles and compared with the CO_2 uptake using bare soil to characterize potential benefits of using rock piles as a mean of bolstering CAM photosynthesis.

MATERIALS AND METHODS

Rock pile field experiment

Experimental set up using rock piles

In the summers of 2018 and 2019, we evaluated the physiological responses of *Agaves* cultivated using rock piles. We analyzed basic characteristics of Hohokam rock pile sites such as size, rock types, number of rocks per pile, diameter, and height in order to build our own similar rock piles (Fig 1.b). Rock piles were built using sedimentary rocks as found at Hohokam rock-pile fields in Arizona (Fish and Fish, 2014; Masse, 1979). The geometric shape and dimensions of the experimental rock piles were calculated using data collected in field surveys made in 2017 and 2018 at Hohokam rock-pile fields from central and southern Arizona. The surveys were done with the assistance of emeritus archaeologists Paul and Suzanne Fish of the Desert Laboratory on Tumamoc Hill. To construct and shape experimental rock piles similar to Hohokam rock piles, we extracted dimensions and shapes from rock piles found at archaeological sites. Conical shape and dimensions of a mound, as reported in field surveys (Masse, 1979; Thompson et al., 2013), can be estimated using basic geometry. The conical mound shape used for our experimental rock piles was calculated using formula (1):

(1) Rock pile shape mound = $\pi\, r\ (r + \sqrt{r2} + h2)$ (Thorp, 1949)

We conducted a composition analysis of rock piles to determine the number and color of rocks and features that we used in our experimental rock piles. Using AgiSoft PhotoScan software, we digitally estimated the area and number of rocks from images collected from Hohokam rock-pile fields to create a model of our rock piles (Figure 3-1a-c). After extracting basic aspects of rock piles (e.g., number and size of rocks), we selected similar rocks found at the BYU greenhouse facility at Brigham Young University, Provo, Utah, to create an experimental rock-pile field.

The experimental rock-pile field was constructed in an open field section of the Brigham Young University greenhouse (Figure 3-2). The dimensions of the rock pile field were 21 m x 8 m and was divided in 8 plot rows of 15 m long. Three rows with rock piles in the north section of the experiment and two rows in the south, west and east side of the experimental plot were used as border plot rows (Fig. 1). The average dimensions of rock piles used to construct the rock piles were 75 cm in diameter and 30 cm in height, which are similar to rock-pile dimensions reported in previous studies (Cantley, 1991; Fish and Fish, 1990). Rock piles were built using sedimentary rocks with varying thickness from 2–10 cm. We used 50±5 rocks per rock pile. Rocks were piled up in a mound shape (Masse, 1979). Rock piles and *Agaves* were separated by 30 cm between rock piles and a distance of 1 m between *Agaves*, as is found in commercial *Agave* plantations, which typically have a density of 3000 plants ha^{-1} (Cervantes Mendívil et al., 2007; Garcia-Moya et al., 2011a).

Agaves cultivated in rock piles and in bare soil were randomized within rows to compare the effect of rock piles on *Agave* CO_2 uptake relative to that of those growing in bare soil. The field was oriented in an east-west orientation to balance shading of plants, the amount of solar incidence on the *Agave* leaves, and CO_2 assimilation in the experiments. Woodhouse *et al.* (1980) and Rangel-Landa *et al.* (2015) suggested that eastward orientation of *Agave* leaves with respect to sun and solar interception increases nocturnal carbon assimilation. Similarly, Cantley (1991) found that Hohokam rock-pile fields at sloped hills predominantly faced a southeasterly direction in central Arizona.

Agave species cultivated in the experimental rock pile field

In the summer of 2018, small two-year-old *A. americana* and *A. murpheyi* plants, which were on average 30 cm high, as reported by Garcia-Moya, et al., (2011) were transported from a commercial wholesale nursery in Phoenix, Arizona (All Season Wholesale Growers, Phoenix, AZ). Raul Puente-Martinez and Wendy Hodgson, curator of living collections and emeritus botanist, respectively, of the Desert Botanical Garden in Phoenix, Arizona, helped confirmed the taxonomic identity of the purchased plants. We selected *A.murpheyi* because it is one of the few species known to not only be cultivated using rock piles, but also domesticated by the Hohokam (Adams and Adams, 1998; Fish and Fish, 2014). Although there is no record of *A. americana* cultivation using rock piles, this specie has been introduced for landscaping purposes in Arizona, and also experimentally tested as a commercial crop in south-central Arizona (Davis et al., 2017). The work by Davis *et al.* (2017) and McDaniel (1985) on *A. americana* suggests that it has potential to be cultivated as crop in Arizona.

Soon after purchase, plants were placed in a greenhouse room, which was set to environmental conditions as described in Table 3-1. Plants were watered weekly prior to being transplanted into the rock-pile field.

Summer transplanting and plant care during winter

Due to limited precipitation early in the summer months of 2018, the rock-pile field was minimally watered using sprinklers to moisture within 10 cm of the soil surface for transplanting. *Agave* plants were transplanted to rock piles and into bare soil to compare nocturnal CO_2 uptake. In the winter of 2018, after finishing measurements the plants were removed from rock piles and repotted in 18.9 L plastic pots using media (pumice, sand, and coconut vermiculite, 3:1:1) and moved indoors to the succulent greenhouse room to protect the *Agave* from night freezing temperatures characteristic of fall and winter in Provo, Utah (Figure 3-3c). The repotted *Agave* were watered weekly to hydrate the plants during the winter season.

In the last week of November of 2018, the rock pile field was covered with commercial 6-mil translucent plastic (Husky, Milwaukee, WI) (Figure 3-3b) to control germination of winter-annual and summer-annual weed species in the plot. In addition, plastic mulching reduced excessive snow moisture underneath rock piles, increasing soil dryness to replicate soil dryness conditions as in south-central Arizona (Figure 3-3c). In 2019 at the end of winter and early spring in April, the plastic mulch was removed to prepare rock piles for harvesting rainwater and transplanting *Agave* in rock piles and bare soil. Rain occurred early in the summer in May of 2019, providing moisture that reached 10-cm saturation depth necessary for transplanting *Agave* plants to the experimental plot (Figure 3-3a). *Agaves* were transplanted to rock piles and bare soil for CO_2 uptake measurements after the second week of May, when the risk of subzero temperatures is generally lower. Measurements in the experiment were made two weeks after transplanting *Agave* into rock piles and bare soil in the summers of 2018 beginning in mid-July and 2019 beginning in the first week of June.

Soil at the experimental rock pile field

Homburg et al. (2011) reported that sandy loam soil types at Hohokam dryland farming sites are a product of soil buildup, which enhanced infiltration, root growth, and water storage of *Agaves*. We used sandy loam soil in our experimental plot to replicate conditions in the Hohokam rock-pile fields as much as possible (Cantley, 1991; Homburg and Sandor, 2011). The nutrient content and drainage of the soil where our experimental rock-pile field was located, which were analyzed at the Enivronmental Analytical Lab, Brigham Young University, Provo, UT, and are summarized in Table 3-2, suggest that the soil exhibits ideal properties for use in cultivating *Agave* with rock piles (Cervantes Mendívil et al., 2007; Fish and Fish, 1990; Sánchez et al., 2020).

Climate at experimental rock-pile field

Agave CAM photosynthesis is highly affected by air temperature and rain (Garcia-Moya et al., 2011b; Nobel, 2003). Using a weather station (ATMOS 41, METER Group Inc. USA, WA), we recorded day and night temperatures and rainfall at the experimental rock-pile field while doing Agave CO2 uptake measurements in order to explore how weather variables impact CO2 uptake of Agave cultivated in rock piles.Moisture content and water potential in rock piles and bare soils.

Weekly water potential and water content was measured in soil samples collected in the rock piles. Water potential was measured in soil samples from the rock piles using a WP4C Dewpoint PotentialMeter (METER Group, Inc, USA). Soil water content at the rock piles was measured using the gravimetric-oven-dry method of Voroney and Sharpe (2019) and calculated using the following formula (2):

(2) Gravimetric soil water content (%) = [mass of moist soil (g) – mass of oven-dried soil (g)/ mass of oven-dried soil (g)] X 100

Soil temperatures in rock piles and bare soils

To measure soil temperatures in the root zones of *A. americana* and *A. murpheyi,* within 20 cm depth we installed thermistors (CS109, Campbell Scientific, Logan, UT), in 5 rock piles and 5 bare soil treatments, which were connected to a datalogger (CR1000X, Campbell Scientific). We also collected soil samples to evaluate temperature and moisture in the rock piles and bare soils at 20–30 cm deep (Nobel, 2002; Thiede, 2020). Soil samples were collected twice a week with a stainless steel 53.3 cm soil sampler (Varomorus, Fl, USA).

Agave leaf temperatures

Using infrared thermal camera model Flir-E5 (Wilsonville, OR, USA) we measured leaf temperature of individual plants in the rock piles and bare soil (Figures 3-4 a and b). The camera was calibrated for collecting pictures at 1 m height. Emissivity of the infrared camera ε was set to 0.95 as estimated for leaf temperature measurements (Jin and Liang, 2006). To ensure pictures were collected at the same height, the camera was attached to a metal tripod. The digital analyses of *Agave* thermal images to extract leaf night temperatures was carried out using the software FLIR Tools version, 5.13 (Wilsonville, OR, USA).

Lab experiment using rock mulching

In order to evaluate the effect of moisture in rock piles on *Agave* CO_2 uptake under temperature-controlled conditions, we designed an experiment with *A. murpheyi* and *A. americana* plants using a 3.9-m^2 controlled-environment growth chamber (GR Series, Environmental Growth Chambers, Chagrin Fall, OH, USA). Lights were programmed to provide a photoperiod of 12-h light/12-h dark at 500 µmol m^{-2} s^{-2}. Based upon temperature data provided by Winter and Holtum (2014) and Nobel et al. (1998), we used temperatures of 35/27˚C light/dark. The goal was to test whether rock mulching could increase CO_2 uptake of *Agaves* under suboptimal conditions. As in the field experiment with rock mulching, we cultivated *Agave* plants growing in rock mulch and bare soils in this experiment.

A total of 24 plants cultivated in rock piles and bare soil were established in a completely randomized design experiment using polypropylene boxes (dimensions: height = 58.4 cm, width = 96.5 cm, depth = 58.4 cm, volume = 0.2 cu m^3 (Figure 3-6). The buckets were filled with sandy-loam soil as in the rock pile field, collected from the Brigham Young Greenhouse facility (Table 3-2). To reduce soil compaction and mimic similar effects of using rock piles for *Agave*, we adjusted the density of the soil to 1.3 g cm^{-3} to replicate soil density in rock piles at archeological sites (Homburg and Sandor, 2011). This experiment consisted of treatments with *Agave* plants that were well-watered and plants in drought. In the well-watered (control) treatment, plants were irrigated applying 1.5 L of water weekly per plant to maintain moisture throughout the experiment. In the drought treatment, plants stop receiving water two weeks prior to CO_2 measurements and did not receive supplemental irrigation for the duration of the experiment.

Agave CO_2 uptake in field and lab experiments using rock piles

In 2018, night CO_2 gas-exchange measurements in the field were made between July and September, beginning on July 14th and ending in September 29th. Using the same procedure for 2018, in 2019, measurements began in June until August, beginning on June 3rd and ending August 30th. Leaf-level photosynthesis measurements were made at a point on leaves of 2–3-

year-old plants, approximately two thirds the distance from the base (Pimienta-Barrios et al., 2001). The dusk-to-early morning between 8:00PM and 9:00AM CO_2 gas-exchange patterns of *A. murpheyi* and *A. americana* were measured in plants growing in rock piles and bare soil. In both years, we used a portable real-time steady-state photosynthesis system (LI-6400XT, LI-COR, Lincoln, NE, USA) (Figure 3-4 a, b). The equipment was modified to make continuous measurements, which enabled the equipment to sample CO_2 uptake of *Agaves* throughout the night over a 12-hr period. The additional components of the LI-6400XT for CO_2 uptake measurements in our experiments are listed in Table 3-3.

Statistical analyses

We used the statistical software SAS Pro 12.6 (SAS Institute Inc., Cary, NC, USA), to perform one-way analyses of variance (ANOVA) to assess statistical differences between CO_2 gas-exchange data of *A. americana* and *A. mupheyi* in rock piles and bare soils in 2018 and 2019. We assessed normality of our response variables using the Shapiro-Wilk test. Using the statistical program JMP Pro 15 (SAS Institute Inc., Cary, NC, USA) none-parametric analysis of variance we assessed differences in soil temperature between rock piles and bare soils. We used the Wilcoxon sum-rank test to analyze soil moisture at different soil depths underneath rocks and bare soils. We conducted correlation analyses between temperature and moisture with CO_2 uptake of *Agave* species in rock piles and bare soils using Sperman correlation analyses.

RESULTS

Agave nocturnal CO_2 uptake in rock piles and bare soil

Rock piles relative to bare soils increased the CO_2 gas-uptake of *Agaves*. Statistical analyses of CO_2 uptake, alpha 95% confidence, indicated differences ($p<0.05$) of CO_2 uptake of *Agaves* cultivated in rock piles and bare soil (Fig. 5 a). Unlike the higher differences in CO_2 gas uptake in rock piles relative to bare soils observed in 2018, the nocturnal CO_2 uptake in 2019 remained very similar between plants growing in rock piles and bare soil (Figure 3-7). The CO_2 uptake of *A. murpheyi* plants in rock piles were higher than plants growing in bare soil in both 2018 and 2019. By contrast, *A. americana* CO_2 uptake was higher in rock piles than bare soils in 2018, but very similar in 2019. Overall, we observed that maximum, mean, and total CO_2 uptake of *A. americana* and *A. murpheyi* were affected by using rock piles. Differences between *Agave* plants in rock piles and bare soil were not observed on the minimum CO_2 uptake.

Maximum CO_2 uptake of Agave in rock piles and bare soil

In 2018, the maximum CO_2 uptake of *A. americana* in rock piles and bare soil statistically differed, by increasing CO2 exchange of *Agave* leaves. Maximum nocturnal CO_2 uptake of *A. americana* in rock piles (16.4 µmol m^{-2} s^{-1}) was over 1.4 times greater than of those in bare soil (11.8 µmol m^{-2} s^{-1}). However, in 2019, the maximum CO_2 uptake of *A. americana* were close between rock piles and bare soil, yielding 14.2 $µmol^{-2}$ s^{-1} in rock piles and in bare soil was 14.2 µmol m^{-2} s^{-1} (Figure 3-7b and Figure 3-8).

The maximum CO_2 gas uptake of *A.murpheyi* plants in rock piles (17.3 µmol m^{-2} s^{-1}) exceeded that of those in bare soil (15.6 µmol m^{-2} s^{-1}) in 2018. Analysis of variance of the maximum CO_2 gas exchange of *A.murpheyi* in rock piles and bare soil showed little differences ($p=0.1$). Minor variations in maximum nocturnal CO_2 uptake were similar in 2019 for *A. murpheyi* cultivated in rock piles and bare soil (Figure 3-8a). *A. murpheyi* plants yielded maximum nocturnal CO_2 uptake of 18.1 µmol m^{-2} s^{-1} in bare soil and 20.8 CO_2 µmol m^{-2} s^{-1} in rock piles (Figure 3-8b). Minimum CO2 gas-exchange did not differ ($p > 0.10$) between *A.murpheyi* plants in rock piles and bare soil.

Mean CO_2 uptake of Agave in rock piles and bare soil

In 2018, mean CO_2 uptake of *A. americana* in bare soil was observed to be 6.3 µmol m^{-2} s^{-1}. By contrast, higher CO_2 uptake of *A. americana* in rock piles was observed at 9.8 µmol m^{-2} s^{-1} (Figure 3-9a). Rock piles increased nocturnal CO_2 mean uptake of *A.americana* by 3.5 µmol m^{-2} s^{-1}. Analysis of means of *A. murpheyi* CO_2 uptake indicated statistical differences between mean *Agave* in rock piles relative to mean CO_2 observed in bare soil ($p=0.05$). Mean CO_2 gas exchange from *A. murpheyi* was roughly two times higher in rock piles than bare soil. Mean CO_2 uptake in rock piles was 9.1 µmol m^{-2} s^{-1} and mean CO_2 uptake of *A.murpheyi* in bare soil was 4.6 µmol m^{-2} s^{-1}.

Different than 2018, in 2019 the mean CO_2 uptake values were close for rock piles and bare soil (Fig. 7a, b); for *A. americana* in rock piles was 9.8 µmol m^{-2} s^{-1} and for bare soil it was 9.5 µmol m^{-2} s^{-1}. For *A. murpheyi,* similar to results in 2018, in 2019 rock piles increased the mean CO_2 uptake (Figure 3-9b). The mean CO_2 gas uptake of *A. murpheyi* growing in bare soil was 5.8 CO_2 µmol m^{-2} s^{-1}. Mean CO_2 uptake in rock piles was observed to be 10. 6 µmol m^{-2} s^{-1}.

Statistical analysis (alpha 95% confidence) indicated differences between nocturnal mean CO_2 uptake in rock piles and bare soil ($p < 0.05$).

Total CO_2 uptake of Agave in rock piles and bare soil

In 2018, the total nocturnal CO_2 uptake of *A. americana* plants was 659.4 CO_2 µmol m^{-2} s^{-1} in rock piles and 362.5 CO_2 µmol m^{-2} s^{-1} in bare soil. Overall, the analysis of the total CO_2 uptake of *A. americana* plants in 2018 indicated that rock piles promoted higher CO_2 uptake in *A. americana* plants throughout the night than in bare soil. However, in 2019, the total nocturnal CO_2 uptake of plants growing in rock piles (657.4 µmol m^{-2} s^{-1}) was similar to that of plants growing in bare soil (621.1 µmol m^{-2} s^{-1}) for bare soil ($P \geq 0.10$) (Figure 3-10).

The mean CO_2 uptake of *A. murpheyi* plants were higher in rock piles than in bare soil; for total CO_2 uptake in both years in 2018 and 2019, the total CO_2 uptake of *A. murpheyi* was higher in rock piles than bare soil. The statistical difference for *Agaves* in rock piles than those cultivated in bare soil was $p<0.05$. The total CO_2 uptake in rock piles was higher in 2019 in rock piles at 692.3 CO_2 µmol m^{-2} s^{-1}. By contrast, CO_2 uptake in bare soil was 301.8 CO_2 µmol m^{-2} s^{-1} (Figure 3-10).

Agave temperatures in rock piles and in bare soil

In 2018, we collected temperatures of single-leaf readings with the LI-6400XT while measuring CO_2 uptake in plants of both *Agave* species. The results showed an increase of maximum temperatures of *A. americana* and *A. murpheyi* plants in rock piles relative to bare soils in 2018 and 2019. However, the minimum temperature for *A. americana* between years was very similar in 2019 as the previous year. However, increases in minimum and mean temperatures of *A. murpheyi* relative to *A.americana* plants growing in rock piles and bare soil in 2019 also occurred (Table 3-4).

Analyses of whole-plant minimum temperatures using thermal images yielded similar values as the LICOR-6400XT, as temperature measurements made when CO_2 gas exchange was measured, which indicated that plant temperatures of both species were 1℃ higher in rock piles than in bare soils in 2018 and in 2019. The mean temperature of *A. americana* plants in rock piles was 14℃ (SD ± 3.5) and 14. 2℃ for *A. murpheyi* plants in rock piles (SD ± 3.5). The mean temperature of *A. americana* plants in bare soil was 13℃ (SD ± 3.0). Likewise, *A. murpheyi* growing in bare soil temperature was 13.2℃ (SD ± 3.2).

Air temperatures and rainfall in 2018 and 2019

Day and night temperatures at the experimental rock pile field were similar in 2018 and 2019. Although *Agave* field measurements were made between mid and late summer in 2018 and early and mid-summer in 2019, the range of temperatures observed were very similar between years. The major climate factor that was different in the experimental season in 2018 and 2019 was rainfall. We observed a dry year in 2018 and a wet year in 2019 (Table 3-5). However, such conditions of dryness and moisture provided conditions that enabled us to observe how rock piles harvested and retained rainfall water and *Agave* CO_2 uptake in rock piles.

Temperature and moisture in rock piles and bare soils

Night temperatures

Night and day temperatures underneath rocks remained similar between 2018 and 2019 (Figures 3-11 and 3-12). Bare soil temperatures were cooler in the night and warmer in the day in 2018 and 2019. Temperatures were warmer in the night and cooler in the day in 2019, and statistically different ($p < 0.05$). Analysis of soil temperatures at 10cm depth indicated significant differences ($p<0.01$). Similarly, soil temperatures at 20cm depth in rock piles and bare soil were significantly different ($p<0.01$). Our results indicated that night temperatures at 10cm depth can be significantly affected by rock piles. The maximum nighttime temperature recorded underneath rock piles was 29.9 ℃ and the minimum temperature underneath rock piles was 21.1 ℃. In 2018, we observed that soil underneath the rock piles were 9.2℃ warmer than bare soil temperatures (Figure 3-11). In 2019, although temperatures underneath rock piles were warmer than bare soils, they did not statistically differ. The maximum temperature of soil underneath rock piles was 34 ℃ for rock piles and 32.8 ℃ in bare soils. The minimum temperature was 26.1 ℃ for rock piles and 24.9 ℃ for bare soil. Overall, compared with bare soils, rock piles warmed the soil 1.2℃.

Day temperatures

In 2018, the maximum bare-soil temperature (56.5℃) was 1.6 times greater than the maximum temperature underneath rock piles (35.1℃) (Figure 3-11). In 2018, the minimum day temperature of soil underneath rock piles (17.7℃) was only 1.2 times greater than the minimum bare-soil temperature (14.7℃). However, in 2019, the maximum daytime rock-pile soil temperature (39.1℃) was only 8.6℃ cooler than the maximum daytime bare-soil temperature (47.7℃) (Figure 3-11).

Water potentials in rock piles and bare soils

Water potential was higher in rock piles (Figure 3-13). The mean soil-water-potential values underneath rock piles were statistically different than in bare soils in 2018 and 2019 ($p<0.005$). The mean soil water potential within 10-cm depth underneath rock piles in 2018 was -1.7 MPa. The mean soil water potential in rock piles in 2019 at 10-cm depth below the surface of the soil, underneath rock piles was -31.7 MPa. Similarly, in 2018 and 2019 statistical differences in soil water potential were found at the 20-cm depth underneath rock piles ($p = 0.05$). In 2018, the mean soil water potential at 20-cm depth underneath rock piles was -0.9 MPa. Conversely, in 2019, mean soil water potential in bare soils at 20cm was -9.9MPa. Fewer negative potentials were found at 30cm depths underneath rock piles. Statistical differences between 2018 and 2019 was calculated at ($p=0.05$). In 2018 the statistical differences of soil water potentials underneath rock piles relative to bare soils was calculated at $p=0.05$ and in 2019 was calculated at ($p<0.01$).

In 2018, the mean soil water potential of bare soil at 10-cm depth was -16.1 MPa. In 2019, the mean soil water potential at 10-cm depth in bare soils was -76.1 MPa. Statistical analysis of the soil water potential means at 10cm indicated differences rock piles compared with bare soils of ($p<0.01$) between 2018 and 2019. Soil mean water potential was less negative in bare soils at 20cm than at 10cm depth in 2018 and in 2019. Soil water potential at 20cm depth between 2018 and 2019 were very similar. In 2018, soil water potential at 20cm depth was -14.0 MPa and in 2019 -17.7MPa. Likewise, in 2018 as in 2019 soil water potentials in bare soils at 30cm depths were less negative and different than in upper soil layers at 20cm and 10cm depths ($p<0.05$). The mean soil water potential at 30cm depth in bare soil was -0.2 MPa. In 2019 mean soil water potential was -8.2MPa. Statistical analysis between mean soil water potentials in rock piles and bare soil in 2018 and 2019 indicated statistical differences ($p<0.0001$).

Soil moisture content in rock piles and bare soils

In July and September 2018, soil moisture content from samples collected in rock piles was slightly higher than in bare soils (Figure 3-14). In July, the soil moisture content in rock piles was 16.9% and 18.8% in bare soil. However, due to precipitation in August, the moisture content was slightly higher in rock piles with 15.4% and 13.5% in bare soil. By the second half of September, the soil moisture content in rock piles was only 6.4% and 16.7% in bare soil.

In 2019, rock piles harvested slightly higher soil moisture content than bare soil; soil moisture content underneath rock piles and bare soil was statistically different ($p=0.03$). During the summer season, moisture was estimated at 9.3% underneath rocks and 8.6% in bare soils. The mean soil moisture content in rock piles was 4% (SD± 2.4). The mean soil moisture content in bare soils was 4.6% (SD± 2.4). Beginning after the second week of May when *Agave* were transplanted into rock piles and soil moisture measurements; rock piles and bare soils measurements were statistically different ($p=0.002$). Soil maximum moisture content was 8.9% in rock piles and 8.6% in bare soils. Mean soil moisture content in bare soils was 6.5% (SD± 1.1). Mean soil moisture content in rock piles in May was 7.4% (SD± 0.7).

Although there was more moisture in soils underneath rock piles than bare soils from June to August, we found no statistical differences. In June, soil moisture content underneath rock piles was slightly higher, but not statistically different than in bare soils ($p=0.3$). The maximum soil moisture content in rock piles was 9.3% with a mean of 5.3 (SD± 1.7). The maximum soil moisture content in bare soil was 7.1% with a mean of 4.5% (SD± 1.8).

In July, soil moisture content in rock piles was 4.9%, with a mean of 2.7% (SD± 1.1); the soil moisture content in bare soil was 4.2% in bare soils with a mean of 1.9% (SD± 1.3). In August, soil moisture content did not differ between rock piles and bare soil (p= 0.2). The soil moisture content in rock piles was 3.9% with a mean of 2.1% (SD± 1.1); the soil moisture content in bare soil was 4.8% with a mean of 1.7% (SD± 1.5).

Relationships of air and leaf temperature with Agave in rock piles and bare soil

Our results indicated that there is relationship between air temperature, leaf temperature, and CO_2 uptake of *A. americana* and *A.murpheyi* (Table 3-6). This relationship affected CO_2 uptakes of *Agave* in bare soil and rock piles. The relationship between air temperature and leaf temperature was observed in *A. murpheyi* in rock piles, but not in plants in bare soil. Similarly, a relationship between air temperatures and leaf temperatures was observed for *A. americana* in exposed soil, but not for rock piles. Likewise, CO_2 uptake was correlated with *A. americana* in rock piles but not for *A. murpheyi*.

Relationship between soil temperature and Agave in rock piles and bare soil

Night soil temperatures were warmer in rock piles compared with bare soil (Figure 3-11). The correlation analyses of nighttime soil temperature in bare soil and rock piles with leaf temperature of *A. americana* indicated that temperatures that occurred within 10 and 20 cm depth correlates with leaf temperature ($p<0.04$). This correlation of temperature suggests a relationship of soil temperature with nocturnal CO_2 uptake. Analyses of correlation between soil temperatures in bare soil with *A. americana* CO_2 uptake indicated that the cool surface soil temperatures correlate with CO_2 uptake ($p=0.02$), but not for rock piles ($p=0.09$). We found slight correlation ($p=0.07$) between soil temperatures in bare soil and rock piles with the total CO_2 uptake of *A. murpheyi*.

Relationship between water potential and Agave in rock piles and bare soil

Correlation analyses of the mean and total CO_2 uptake of *A. americana* in bare soil indicated statistical correlation with the water potential at 10cm depth in bare soil ($p < 0.04$). In contrast, we did not observe correlation of water potential within 20 cm depth and nocturnal CO_2 uptake ($p > 0.1$). Bare-soil water potential within 10 and 20 cm did not correlate with nocturnal CO2 uptake of *A. murpheyi* ($p \geq 0.1$). However, water potential within 10 and 20 cm depth in soil underneath rock piles were highly correlated ($p<0.01$) with nocturnal mean and total CO_2 uptake.

Lab experiment: Agave CO_2 uptake in rock piles and bare soil

The analysis of mean CO_2 uptake of *A. americana* in bare soils indicated statistical differences ($p < 0.0001$) between well-watered and low-water conditions (no irrigation while doing the experiment). The CO_2 uptake of well-watered and low-water *A. americana* plants in rock piles differed ($p < 0.002$). Similarly, well-watered *A. americana* in rock piles and in bare soil were statistically different ($p=0.02$). However, well-watered *A. americana* cultivated in rock piles and low-water rock piles showed no statistical differences between CO_2 uptake ($p=0.2$).

We observed no differences ($p=0.5$) between CO_2 uptake of well-watered *A. murpheyi* and *A. murpheyi* under low-water conditions. In addition, we found statistical differences ($p< 0.001$) between the CO_2 uptake of *A. murpheyi* in rock piles that were well-watered and plants in dry conditions. Similarly, statistical differences ($p<0.001$) were observed for CO_2 uptake of well-watered *A. murpheyi* in rock piles and bare soils.

DISCUSSION

Agave CO_2 uptake in rock piles

Rock mulching promoted higher CO_2 nocturnal uptake of *Agave* than uptake in bare soils. We observed that CO_2 uptake can be favored by the insulative properties of rock piles. Despite seasonal changes in day/night temperatures and rainfall in our experiments between 2018 and 2019, rock piles functioned as a soil insulator, rock piles blocked heat transference, keeping cooler soils during the day and reduced heat dissipation in the night (Figures 3-11 and 3-12). Our observations using rock piles coincide with previous studies that indicated the benefit of using rock piles, in which they observed that rock piles increased soil moisture, reduced soil moisture evaporation, and insulated soils underneath rocks (Brevik et al., 2016; Cui and Nobel, 1992; Homburg and Sandor, 2011; Nobel et al., 1992). Little amounts of soil moisture content and mild air and soil temperatures increases nocturnal CAM photosynthesis of *Agave* (Garcia-Moya et al., 2011b, 2011b; José-Jacinto and García-Moya, 1995; Neales, 1973; Nobel, 1991; Nobel and Quero, 1986; Winter et al., 2014). The main strategy for *Agave* photosynthesis concentrates in the CAM Phase I, in which stomata opens for nocturnal CO_2 uptake and fixing carbon in *Agave* leaf cells (Matiz et al., 2013; Neales, 1975; Niechayev et al., 2019; Nobel, 1977; Winter et al., 2014). The nocturnal CO_2 uptake of *Agave* in our experiment are the product of the water use efficiency of *Agave* and CAM photosynthesis of *Agave*, but also the product of the moisture content, water potentials, and temperatures underneath rocks in 2018 and 2019 (Figures 3-7, 3-11 to 3-14).

Among the many benefits of using rock mulching to cultivate *Agave* are the low temperatures underneath rocks in the day and warm temperatures in the night (Nobel et al., 1992) (Figs. 9 and 10) and also the reduction of soil moisture evaporation rates (Brevik et al., 2016; Homburg and Sandor, 2011; Nobel et al., 2002; Wilken, 1972). *Agave* has been widely reported to adapt to heat and drought and continue photosynthesizing beyond a wilting point of -1.5 even during rainless years in arid regions because of its water use efficiency strategy, principally using CAM photosynthesis (Eguiarte et al., 2021; Ehrler, 1983; Stewart, 2015). Although some *Agave* species have the inherent capacity to endure long periods without water and high temperatures, slight changes in soil temperatures and water content promoted higher *Agave* nocturnal CO_2 uptake using rock piles in our experiments (Figures 3-7, 3-11,3-12), as indicated by (Ehrler, 1983; Nobel et al., 1994). Cultivating *A.americana* and *A.murpheyi* using two cultivation methods—rock piles and bare soil (Fish and Fish, 2014; McDaniel, 1985)—enabled us to see how rock piles modify microclimate conditions, principally soil temperature and moisture around *Agave* (Figures 3-7 to 3-14).

A.americana and *A.murpheyi* use CAM photosynthesis as the main strategy to photosynthesize in rock piles and in bare soils (Figure 3-7). Field studies made by Nobel et al. (1987) and Nobel and McDaniel (1988) reported that when moisture is available, *A.americana* could shift between CAM and C_3 photosynthesis. Although moisture was available for longer periods of time in rock piles than in bare soils, we observed CAM photosynthesis in both species in 2018 and 2019 (Figure 3-7). The CO_2 uptake patterns we observed throughout the night in rock piles and bare soils of *A.americana* and *A.murpheyi,* are similar to nocturnal CO_2 exchange of other experiments and field observations made using *A. angustifolia*, *A. tequilana,* and *A. salmiana* in dry regions from Mexico (Garcia-Moya et al., 2011). The *A. americana* CO_2 uptake in rock piles and bare soil we observed are similar to CO_2 uptake reported using irrigation experiments cultivating *A. americana* in the Sonoran Desert in Arizona in which Davis et al. (2017) observed nocturnal CO_2 uptake of three-year-old *A. americana* plants at between 11 and 17 μmol CO_2 m^{-2} s^{-1}.

Although rainfall was the primary source of soil moisture during our study, the CO_2 uptake of *Agaves* (Figure 3-7) are comparable to that of irrigated *A. americana* in central Arizona (Davis *et al.* 2017). The maximum CO_2 uptake observed in *A. americana* rock piles in 2018 and 2019 were 16.4 CO_2 μmols and 14.2 CO_2 μmols, respectively. José-Jacinto and García-Moya (1993) reported that, in the summer months in the climate of central Oaxaca, Mexico, CO_2 uptake of *A.angustifolia* was 14 μmol CO_2 m^{-2} s^{-1}. Their observations indicated that night temperature strongly influenced CO_2 uptake in *A.angustifolia*. Likewise, Eickmeier (1979) reported that CO_2 uptake of *A. lechuguilla* can be 6.3 μmol m^{-2} s^{-1} of CO_2 under field conditions in the Chihuahuan desert. Nobel and McDaniel (1988) reported that, under field conditions in southern Arizona, *A. vilmoriniana,* expressed a maximum CO_2 uptake rate of 5 CO_2 m^{-2} s^{-1}. Pimienta-Barrios et al. (2005) reported that CO_2 uptake of *A.tequilana* ranged 9–10 μmol CO_2 m^{-2} s^{-1} in the summer months in Jalisco, Mexico.

In 2018, we observed that rock piles increased the CO_2 uptake in *A.americana* and *A. murpheyi* plants relative to conspecific plants in bare soil (Figs 3-9 and 3-10). Rock piles boosted the CO_2 uptake of *A. murpheyi* compared with the uptake of plants growing in bare soil (Figures 3-9 and 3-10). In 2019, *A. americana* CO_2 uptake, yielded very similar nocturnal maximum and mean CO_2 uptake between rock piles and bare soil (Figure 3-7b). However, *A. murpheyi* CO_2 uptake in rock piles exceeded that of conspecific plants in both 2018 and 2019. The differences observed in CO_2 uptake of *A. americana* is likely due to its sensitivity to small changes of temperature and moisture, particularly water potentials below -9.0, that can reduce CO_2 uptake of *A. americana* (Ehrler, 1983). Shakeel et al. (2013) indicated a small increase of temperature in *A. americana* leaves may reduce stomatal opening activity, impairing CO_2 uptake. In addition, José-Jacinto and García-Moya (1993) suggested that cellular thermal capacity of *Agave* leaves modulates quick adaptations to air night temperatures, as shown by slight changes in *Agave* leaf temperatures between 2018 and 2019 (Table 3-4).

Air temperatures and Agave CO_2 uptake

Nobel (2003) reported that *A. murpheyi* uses CAM photosynthesis as its main water use efficiency mechanism. Likewise, *A. americana* has been widely reported to use CAM photosynthesis as its main photosynthetic strategy (Nobel et al., 2002). High night air temperature can inhibit CO_2 uptake of *A. americana* (Neales, 1973). A study by Winter et al. (2014), and Neales. (1973) observed that high night air temperatures induce stomatal closure and reduce CO_2 uptake of *A. angustifolia* and *A. americana*.

Nighttime temperatures during our study were relatively low, ranging between 13˚C and 20˚C (Table 3-5). These temperatures as observed in our experimental rock pile field can induce optimal stomatal opening and promoting high CO_2 uptake as reported for *Agave* CAM photosynthesis expression (Graham and Nobel, 1996; Holtum and Winter, 2014; Nobel et al., 2002; Pimienta-Barrios et al., 2005, 2001). Low night temperatures likely contributed to increased CO_2 uptake of *A. murphyei* and *A. americana* grown in rock piles. Although Davis et al. (2017) suggested that physiological response of *Agave* tested outside their cultivation and natural range can be vary due to temperatures, several studies have found that mild cool temperatures between 10 and 20 °C can result in increased CO_2 uptake of *Agaves* (Garcia-Moya et al., 2011b; Neales, 1973; Nobel, 2003; Winter et al., 2014). The combination of moderate cooler air temperatures and the use of rock mulch likely promoted the relative high CO_2 uptake we observed in rock piles (Figs 3-7 to 3-10).

Soil temperatures, moisture in rock piles and Agave CO_2 uptake

Rock piles insulated soils from day hot and night cool temperatures (Figs 9–12). The temperature and moisture underneath rocks correlated with nocturnal CO_2 uptake of *Agave* in our experiments (Figures 3-11 to 3-14). Drennan and Nobel (1996) observed that by insulating soils using rocks roots increased growth. *Agave* in rock piles likely experienced less heat and drought stress. Soil high temperatures can be detrimental for productivity and carbon uptake, as reported by *Winter et al*. (2014). Nobel (2003) reported that temperatures in arid regions such as the Sonoran Desert can reach 70 to 80°C during the day. Soil temperature and moisture play an important role in young *Agave* plants, producing biomass of young *Agave* plants and nocturnal carbon fixation (Franco and Nobel, 1989; Garcia-Moya et al., 2011b; Nobel et al., 1992). Even small rocks (diameter = ~5 cm) above *Agave* roots modify soil heat transference about 0.4 °C, which increases roots growth, root microbial colonization, and hydraulic conductivity due to cool temperatures underneath rocks (Cui and Nobel, 1992; Nobel et al., 1992). We observed similar benefits using rock piles: rock piles reduced heat transference during the day and warmed soils in the night, reflecting in increased CO_2 uptake of *Agave* (Figures 3-11 and 3-12).

Young *Agave* (height = ~30 cm) concentrates 16% of dry weight in the root system (Garcia-Moya et al., 2011). In our experiment using similar sized *Agave* plants, the use of rock piles promoted a microclimate for the root zone that also favored CO_2 uptake of *Agave* (Cui and Nobel, 1992; Nobel et al., 1992; Wilken, 1972). Slightly warm night temperatures observed in rock piles has been reported to increase performance of *Agave* CO_2 uptake (Holtum and Winter, 2014). Although *Agave* is a drought tolerant plant and physiologically can function beyond wilting point -1.5 (Ehrler, 1983), we observed that that regardless of the specie, the less negative water potentials in rock piles (Figure 3-13) increased CO_2 uptake of *Agave* (Ehrler, 1983). Small water inputs and reduced water evaporation in rock piles are important for performance and recovering after extended dry periods, as observed for *A. americana* by (Ehrler, 1983). Indeed, little moisture beneath rock piles was associated with increased CO_2 uptake of *A. murpheyi* and *A. americana* plants (Figure 3-13).

CONCLUSIONS

As the risk of drought and heat waves increases in arid regions and threatens irrigated crops, new cultivation methods are needed to continue growing *Agave.* Rock mulching is an alternative method that can reduce irrigation water required to cultivate *Agave* in arid regions. Using rock piles as a dryland farming system to cultivate *Agave* has the potential to conserve resources in hot and dry climates. Our study indicates that rock piles are a feasible method to insulate soils from high temperatures and to cultivate young *Agave* in regions with limited rainwater. As new findings of pre-Columbian *Agave* anciently cultivated in rock piles emerges, our understanding about the application of rock mulching to modern *Agave* cultivation will also expand.

In our experiment, *Agaves* planted in rock piles were exposed to less heat during the day, warmer temperatures in the night, and high moisture levels underneath rocks; these conditions in rock piles promoted higher CO_2 uptake. Rock piles preserved moisture for a longer period of time than bare soil. Insulation of rock piles promoted cooler temperatures during the day than in bare soil; rock piles promoted warmer temperatures at night than in bare soil. Night soil temperatures underneath rocks positively affected *Agave* CO_2 uptake. Despite dry conditions, the little moisture, and temperatures under rock piles increased the CO_2 uptake. In addition, the insulation effect of rock piles remained constant between wet and dry years. The summer dry and wet conditions affect CO_2 uptake in rock piles of *A. americana*. The gain in CO_2 uptake of *A. murpheyi* in rock piles remained higher in rock piles than in bare soil, regardless of summer dry and wet conditions. The benefits of using rock piles on CO_2 uptakes of *A. americana* and *A.*

murpheyi can be observed even when *A. americana* and *A. murpheyi* are induced to their lowest performance conditions.

Experimental gardens and long-term experiments with rock piles and *Agaves* are necessary at different locations in southern-central Arizona and northern Mexico. More species beyond *A. americana* and *A. murpheyi* need to be tested using rock piles; experiments with rock piles and new pre-Columbian domesticated *Agave* species and hybrids found in the region will add understanding for applications of rock piles.

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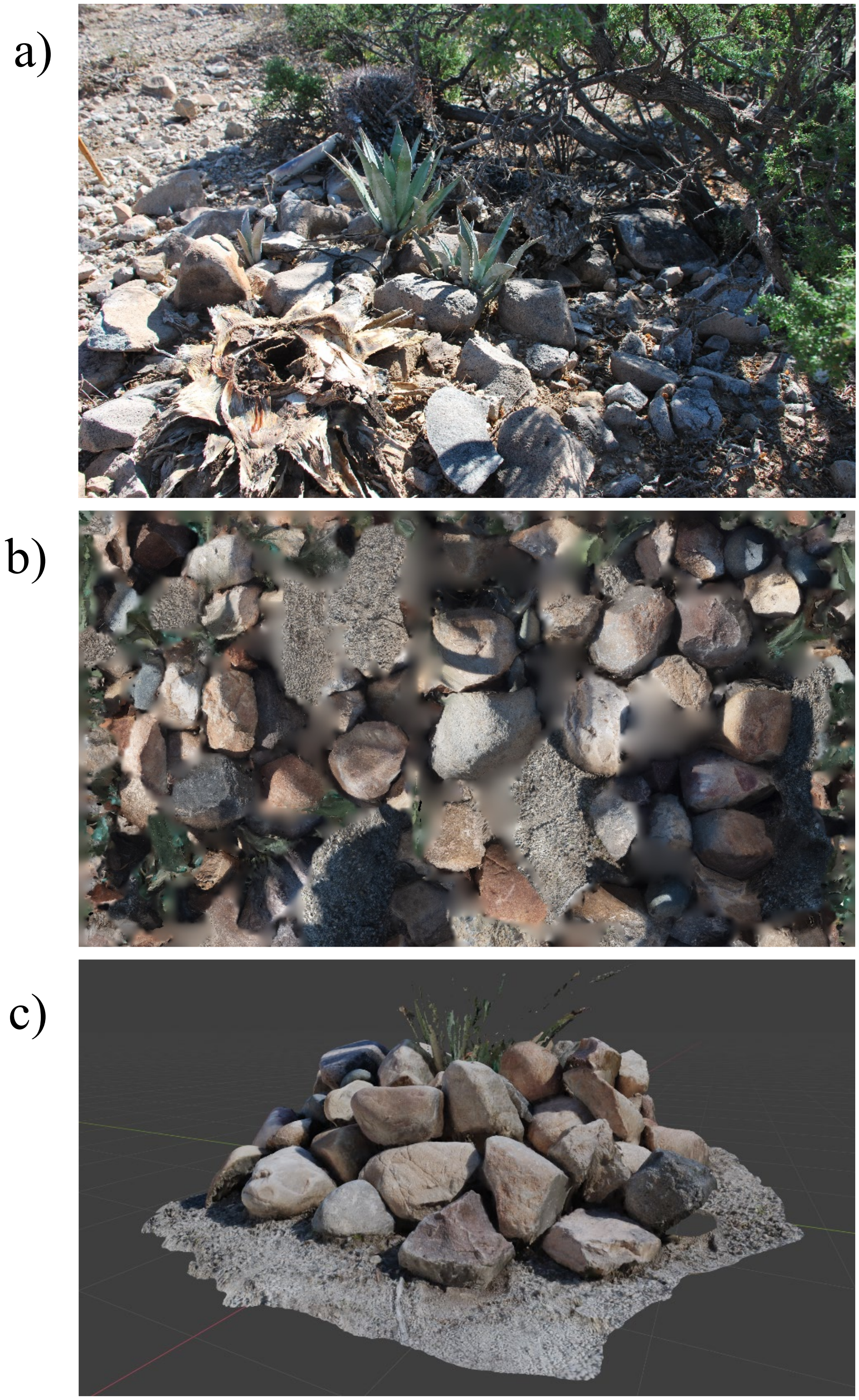

Figure 3-1. Analysis of rock piles to construct an experimental rock piles, (a) Hohokam rock pile at Tumamoc Hill, (b) decomposition and analysis of rocks, (c) 3D experimental rock pile.

Figure 3-2. Experimental rock pile field at Brigham Young University.

a)

b)

c)

Figure 3-3. (a) *Agave* transplanting in rock piles and bare soils 2019, (b) rock pile field covered with plastic mulch early winter 2018, (c) rock pile field in the winter covered with snow.

a) b)

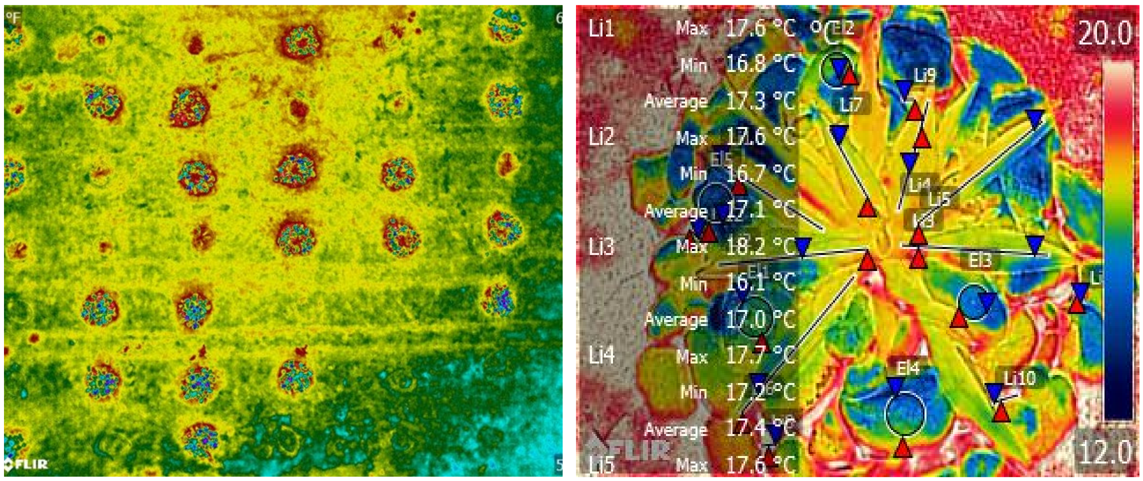

Figure 3-4. Thermal images of rock piles at experimental rock pile field with *Agave*, (a) aerial thermal image of rock pile field with *Agave*, courtesy of Dr. Scott Ure at the BYU anthropology department, (b) thermal image of rock pile with *Agave*.

a) b)

Figure 3-5. CO_2 uptake field measurements using portable infrared gas analyzer LI-6400XT at experimental rock piles: (a) *A. americana* and (b) *A. murpheyi.*

Figure 3-6. Experimental set up in controlled environment chamber at Brigham Young University.

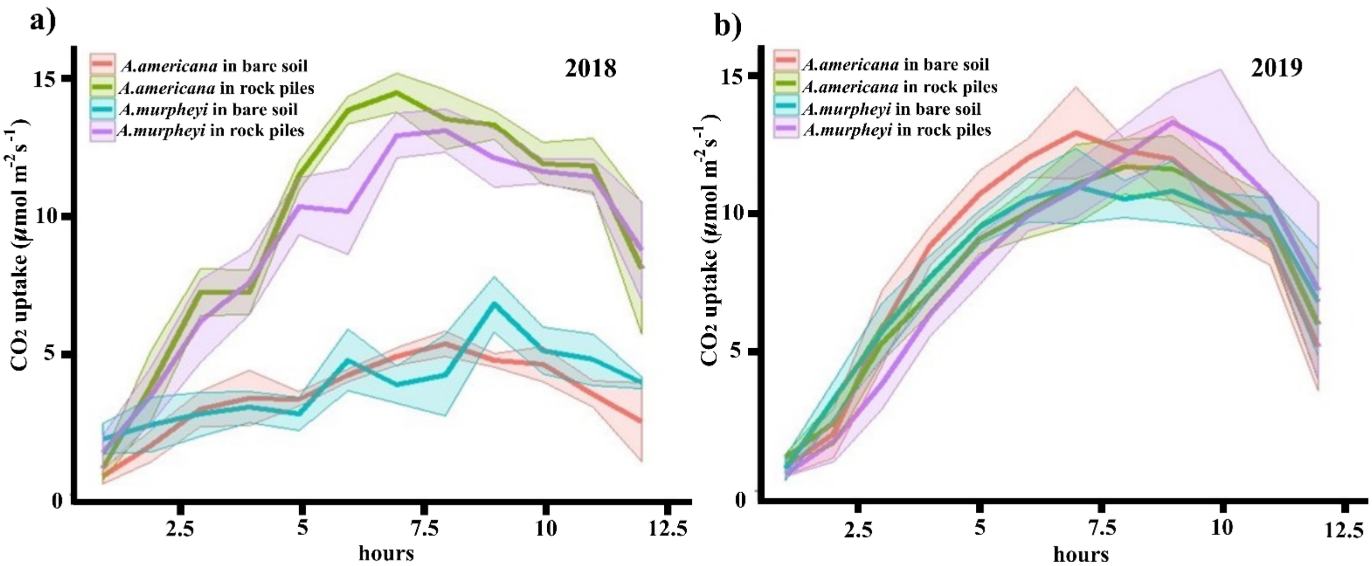

Figure 3-7. CO2 uptakes of *A. americana* and *A. murpheyi* collected from dusk to dawn in rock piles and bare soil at experimental rock pile field at Brigham Young University in two years, (a) 2018 and (b) 2019 .

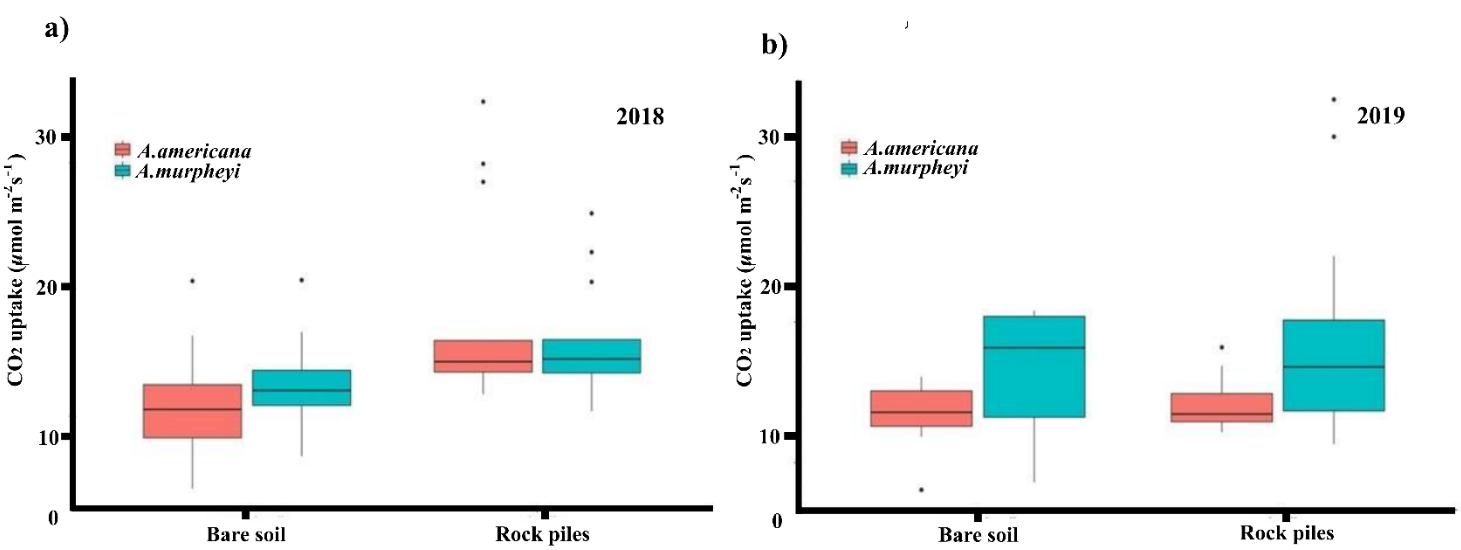

Figure 3-8. Maximum CO_2 uptakes of *A. americana* and *A. murpheyi* in rock piles and bare soil in (a) summer of 2018 Jul–Sept and (b) 2019 Jun–Aug.

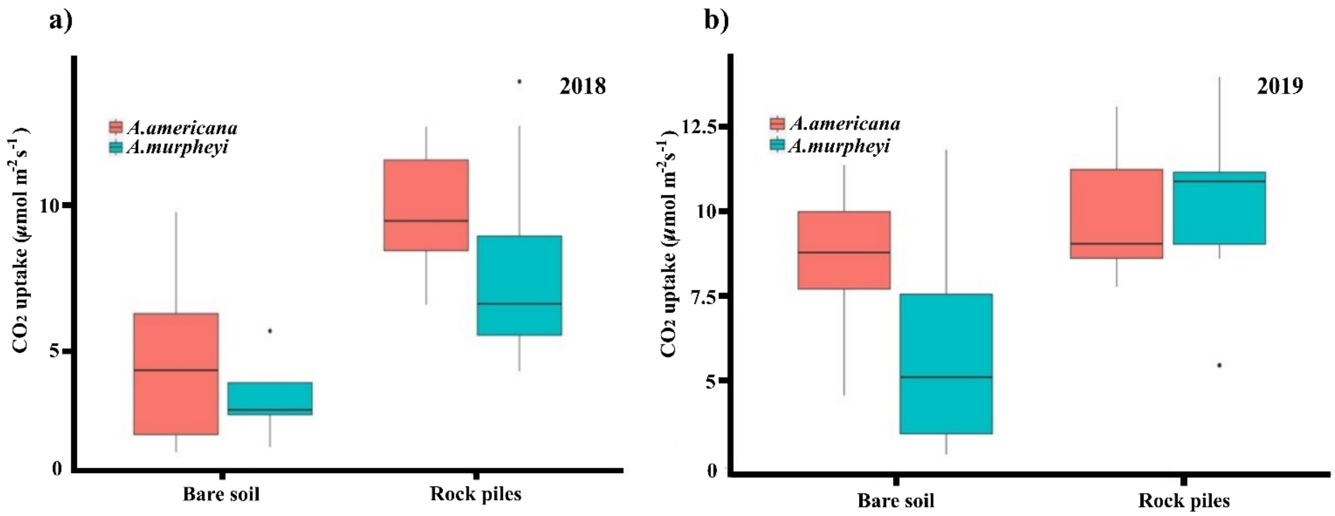

Figure 3-9. Mean CO_2 uptakes of *A. americana* and *A. murpheyi* in rock piles and bare soil in (a) summer of 2018 Jul–Sept and (b) 2019 Jun–Aug .

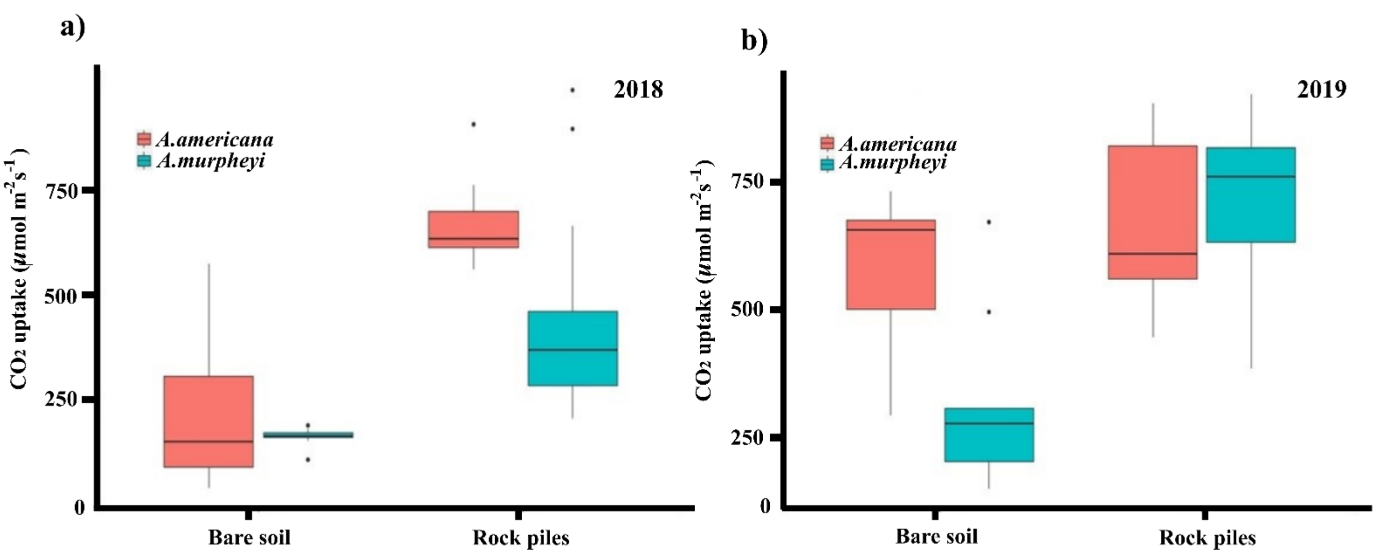

Figure 3-10. Total CO_2 uptakes of *A. americana* and *A. murpheyi* in rock piles and bare soil in (a) summer of 2018 Jul–Sept and (b) 2019 Jun–Aug.

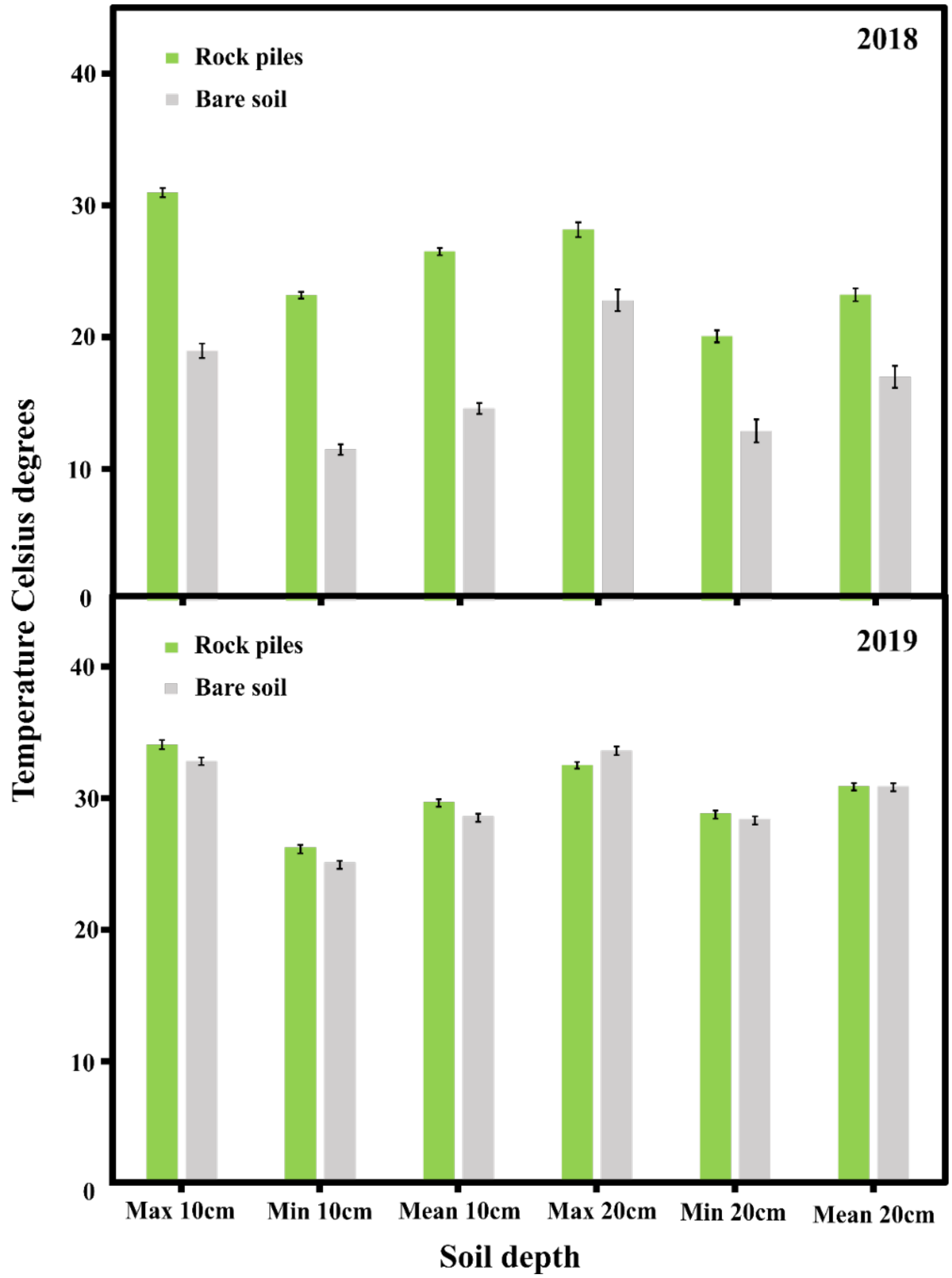

Figure 3-11. Night temperatures underneath rock piles and bare soils in 2018 and 2019.

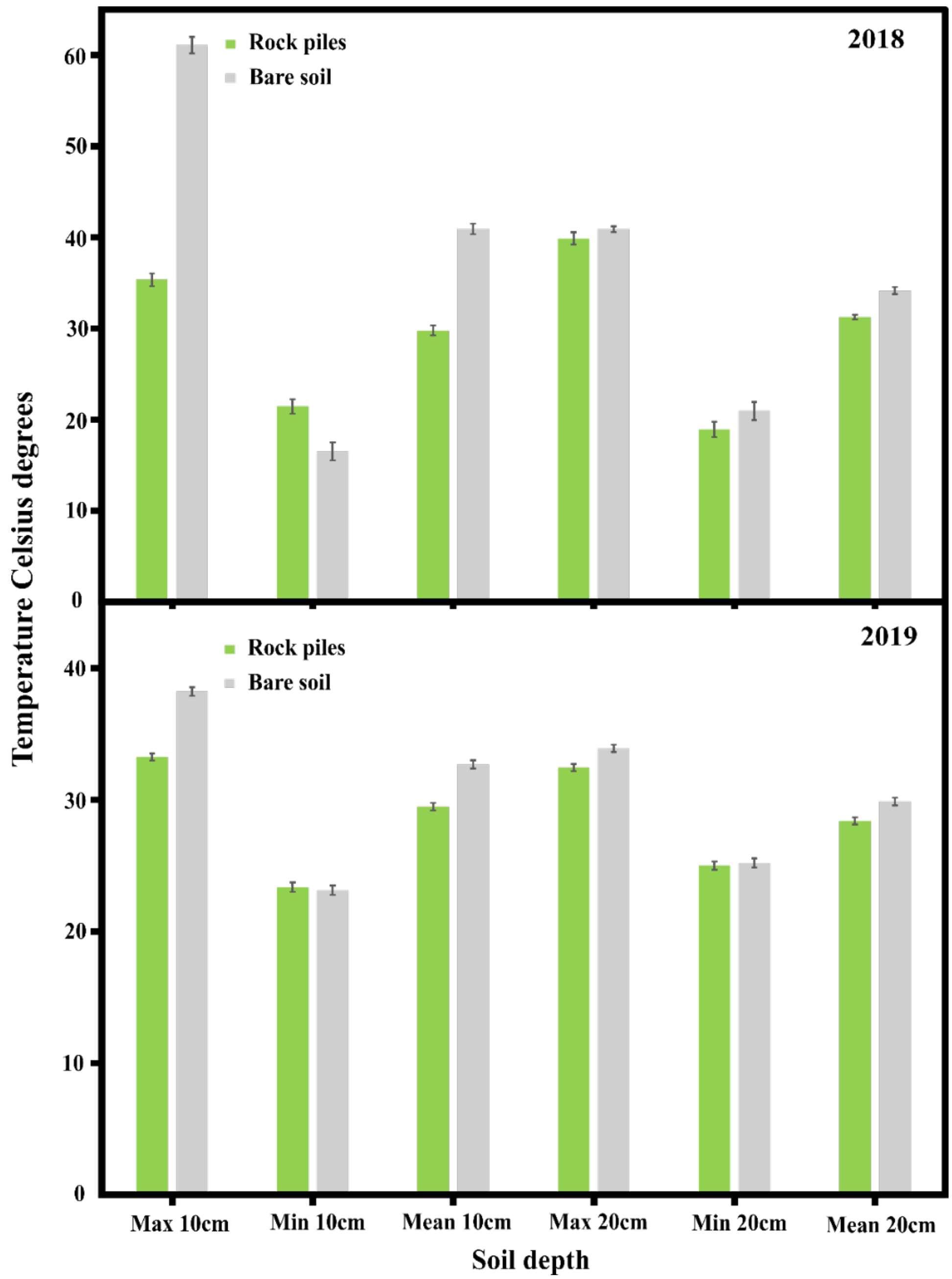

Figure 3-12. Day temperatures underneath rock piles and bare soils in 2018 and 2019.

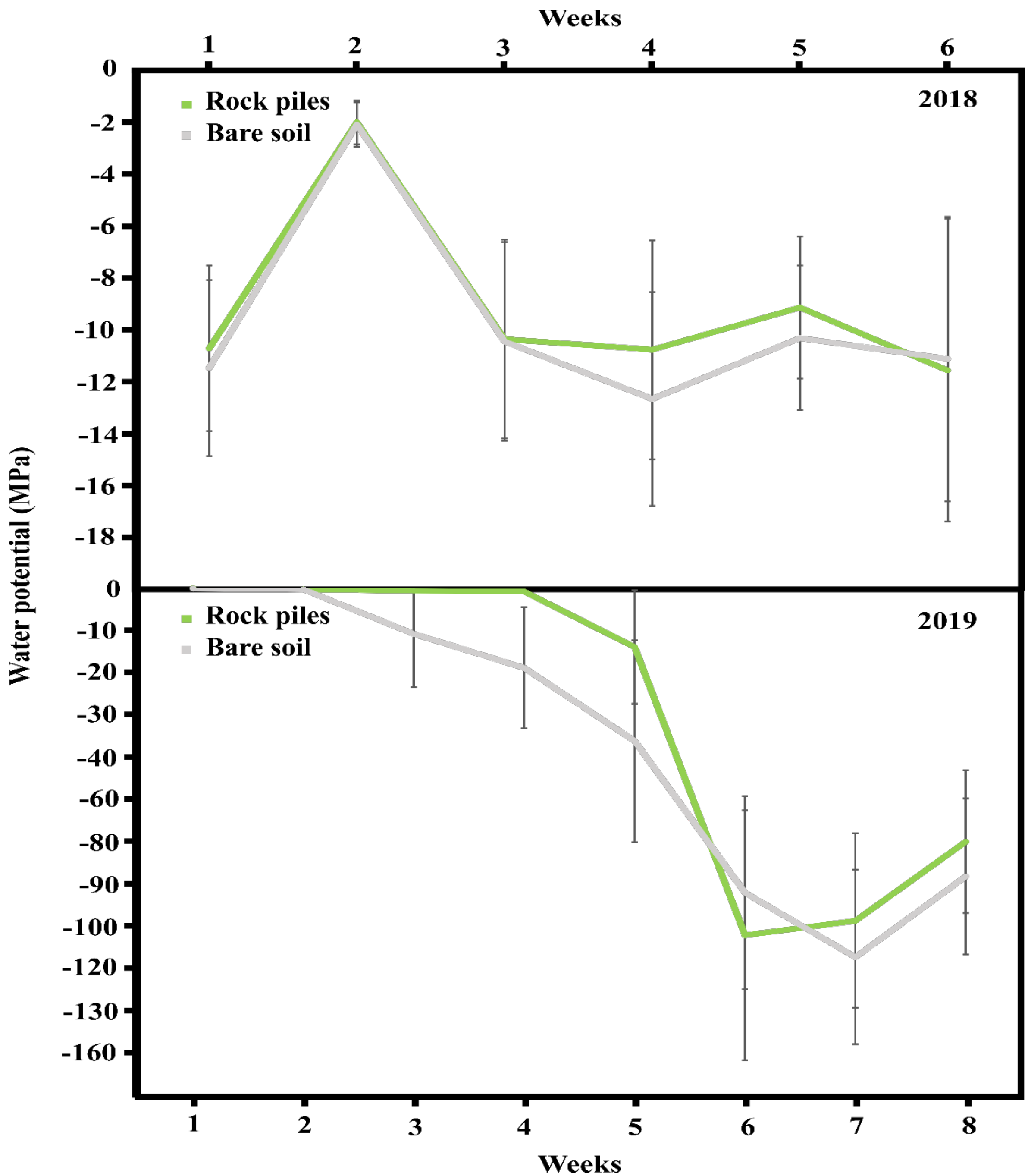

Figure 3-13. Weekly soil water potential values of rock piles and bare soils (a) 2018 and (b) 2019.

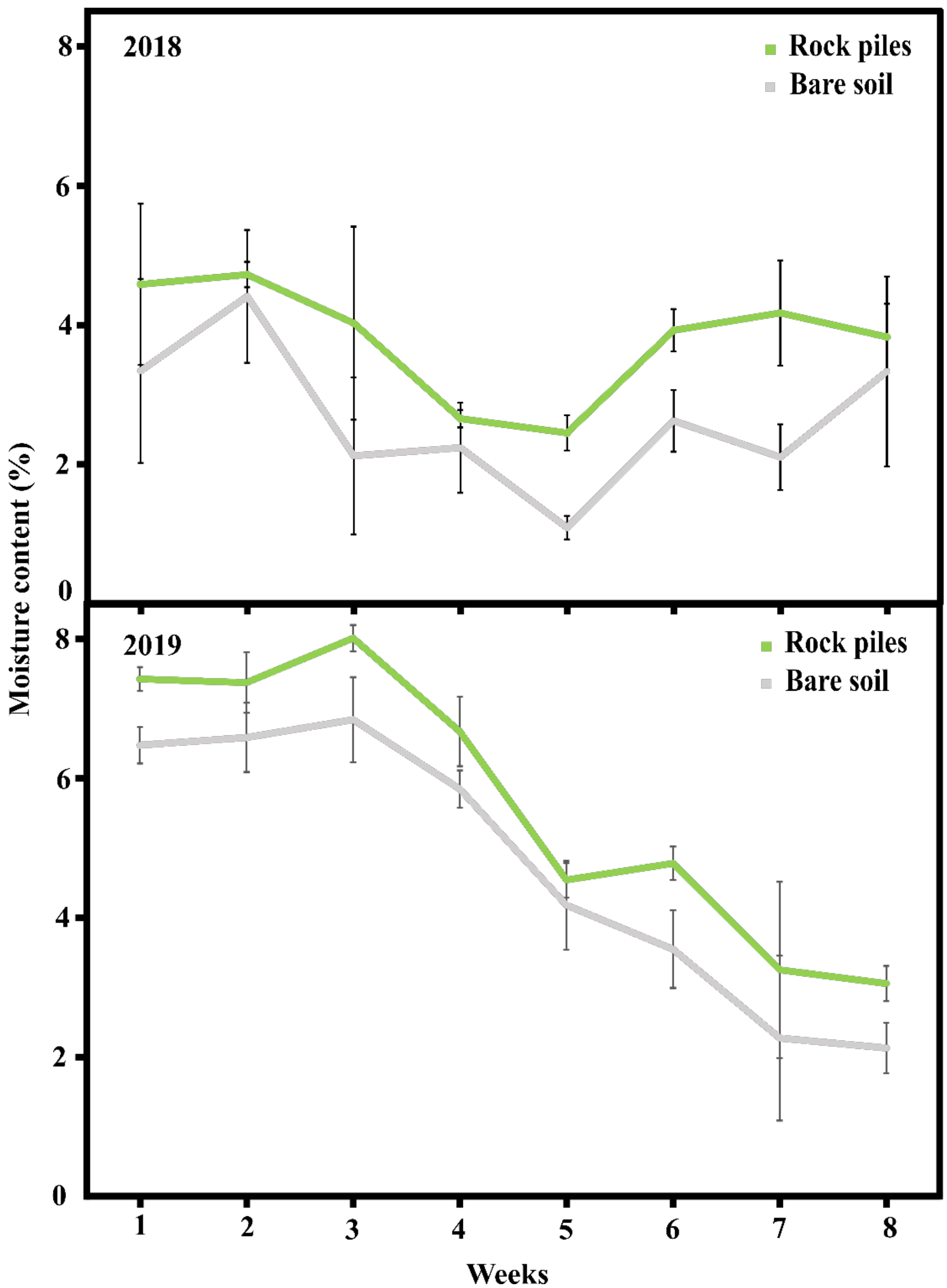

Figure 3-14. Weekly soil water content of rock piles and bare soils (a) 2018 and (b) 2019.

TABLES

Table 3-1. Brigham Young University greenhouse succulent room conditions.

Greenhouse conditions	Day	Night
Heat temperatures	29.5 ℃	24 ℃
Cool temperatures	24 ℃	24 ℃
Relative humidity (RH)	45%	50%
Photosynthetic active radiation (PAR), 12hours	800–1000 µmol s^{-2} cm^{-1}	12 h dark

Table 3-2. Summary of micro and macro-nutrients of the soil where the experimental rock pile field was built and history of the experimental plot.

Soil textura	**pH**	**Cation exchange meq/100g**	**% Organic matter**	**Crop plot history**	**Weeds plot history**	**Watering plot history**
Sandy-Loam	7.7	5.79	0.8 (low)	Agave-Opuntia (3-5 years before rock pile field experiment)	*Ipomea* sp., *Tribulus terrestris, Centaurea solstitialis*	Fertigation system (disabled three years before the rock pile field was established)
NPK-salinity	**Concentration**	**Low**	**Medium**	**High**	**Very High**	**Hazards**
Phosphorus (P) ppm	17	X				
Potasium (K) %	>2					
Salinity-ECe dS/m	0.5	X				No salinity problems
Total Nitrogen (N) %	0.01	X				
Total Carbon (C) %	1.4	X				

Table 3-3. Components of the external CO_2 source that were attached to the LI6400XT to measure CO_2 uptake of *Agave*.

Components	**Manufacturer**
87 cu. Fut capacity aluminun cylinders with 99.9 % CO_2	(Praxair, Salt Lake City, UT)
Tank block connector (9964-033)	(Li-Cor, Lincoln, NE, USA)
2 stage, general purpose brass regulator 3500 PSI inlet/ 400 PSI outlet	(Airgas, Radnor, PA)
General purpose brass regulator 3500 PSI inlet/ 400 PSI outlet	(Praxair Technology, Inc., Danbury, CT, USA)

Table 3-4. Summary of *Agave* leaf temperature in rock piles and bare soil in 2018 and 2019.

Specie,cultivation method	**2018**			**2019**		
	Max	**Min**	**Mean**	**Max**	**Min**	**Mean**
A.americana in rock piles	26.3	16	19.5	29.3	15.8	19.5
A.americana in bare soil	25.4	16	19.4	27.7	16.2	19.4
A.murpheyi in rock piles	27.1	16.1	19.3	30.7	17.3	21.1
A.murpheyi in bare soil	25.1	14.4	17.6	20.5	17.9	21.2

Table 3.5. Summary of air temperature and rainfall in 2018 and 2019 at the experimental rock pile field at Brigham Young University.

Climate	2018				2019			
	Max	Min	Mean	Stdv	Max	Min	Mean	Stdv
Night temperatures warmest	33	27.4	31.6	1.8	32.9	29	31.5	1.1
Night temperatures coolest	17.8	12.5	15.1	1.4	16.4	10	13	1.9
Day temperatures Warmest	35.9	33.4	34.6	0.7	36	33.4	34.8	0.8
Day temperatures coolest	16.6	7.1	12.8	3.4	21	10.1	15.2	3.1
Rainfall (mm)	18.3	0.1	*0.01	3.2	96.6	0.01	*1.2	3.2

*Mean rainfall are expressed in daily mean precipitation throughout the experimental season

Table 3-6. Analysis of correlation between air temperatures with *Agave* temperatures and CO_2 uptake in rock piles and bare soil.

Specie - cultivation method	**Correlation**	**p value**
A.americana in bare soil	Leaf temperature with minimum air temperatura	0.03
A.americana in bare soil	Night maximum air temperature with mean CO_2 uptake	0.04
A.americana in rock piles	Night maximum air temperature with minimum CO_2 uptake	0.04
A.murpheyi in bare soil	Leaf temperature with night maximum air temperatura	0.01
A.murpheyi in rock piles	Minimum and mean leaf temperature with maximum and minimum air temperatura	<0.07
A.murpheyi in bare soils	Mean air temperatures with minimum CO_2 uptake	0.04