Department of Spatial Sciences

Spatial Variability in High Value Crops – Initially Focused on Truffle Production in the South-West of Western Australia

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

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ABSTRACT

Due to its aromatic quality, rarity and difficulty in cultivation, the Périgord black truffle (*Tuber melanosporum*) is a highly valued commodity worldwide. Despite its value to both local and regional economies, the knowledge surrounding truffle cultivation within Western Australia is not greatly understood. Current scientific knowledge estimates that certain soil properties (such as soil classification, pH, drainage / structure (aeration), trace elements and nutrient ratios), and certain climatic conditions may trigger fruiting and impact growth of the black truffle. Currently, very limited research has been conducted to explore which factor(s) have the highest influence over truffle yield.

Using an industry leading black truffle truffière located in the south-west of Western Australia, this research intends to explore spatial variability in truffle yield. Various spatial analysis methods, such as the *Average Nearest Neighbour Ratio* and *Optimised Hot Spot Analysis*, will be utilised to describe and map the spatial distribution of harvested truffles over the 2019, 2020 and 2021 harvest seasons. This spatial distribution will then be examined against various soil properties, obtained through a site soil sampling survey, to determine which factor(s) have highest influence over truffle yield.

This research found that loamy sand soils within the Site B study area were generally too moist and too acidic to facilitate a high level of production. Over the 2019 and 2020 harvest seasons, high truffle yield was generally found in sample locations containing low moisture contents (%), copper (mg/kg), nitrogen (%) and sulphur (mg/kg) content values. Truffle yield increases as both pH and phosphorous content (mg/kg) values increased. These relationships were confirmed through the *Exploratory Regression* tool. The *Ordinary Least Squares* tool further confirmed these relationships and suggested that nitrogen and copper content were the only 2 properties that statistically influenced truffle yield over the 2019 harvest season.

This study only provides a basic understanding of the influence of soil properties over truffle yield. In order to determine which soil property / properties have the most influence over yield, and the extent of these relationships, a soil scientist with a greater understanding of soil chemistry is required. Additionally, a soil scientist may assist in identifying whether it is a combination of soil properties, rather than just a single one, that work in symbiosis with each other to produce a higher yield of truffles. Findings from this research aim to improve both the utilisation of resources and potentially increase harvest within the truffière, in order to maintain the economic benefits to both the study truffière and the wider south-west region.

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1 INTRODUCTION

Due to its aromatic quality, rarity and difficulty in cultivation, the Périgord, or as it is more commonly known, the black truffle (*Tuber melanosporum*) is a highly valued commodity worldwide (Bradshaw, 2005). Despite its value to both local and regional economies, knowledge surrounding truffle cultivation within Western Australia is not greatly understood. Current scientific knowledge estimates that certain soil properties, such as soil classification, pH, drainage / structure (aeration), trace elements and nutrient ratios; and certain climatic conditions may trigger fruiting and impact growth of the black truffle. Currently, very limited research has been conducted to explore which factor(s) have the highest influence over truffle yield.

Using an industry leading black truffle truffière located in the south-west of Western Australia, this research intends to explore spatial variability in truffle yield. Various spatial analysis methods, such as Hot Spot Analysis, will be utilised to describe and map the spatial distribution of harvested truffles over the 2019, 2020 and 2021 harvest seasons. This spatial distribution will then be examined against various growing factors to determine which climatic conditions (rainfall and temperature levels) or soil factors (pH, moisture content, structure and nutrient ratios), if any, have the highest influence over truffle yield. Findings from this research aim to improve both the utilisation of resources and potentially increase productivity within the truffière, in order to maintain the economic benefits to both the study truffière and the wider south-west region.

1. 1 The Australian Truffle Industry

The Australian black truffle industry was first established in Tasmania in the early 1990s, and Western Australia in 1997, with the first Australian grown commercial black truffles harvested by 1999 and 2003 (Hall & Haslam, 2012; Rural Industries Research and Development Corporation (RIRDC), 2014; Trufficulture Pty Ltd., n.d.). By 2007, two-thirds of Australia’s black truffle production had shifted to Western Australia, specifically to the Manjimup and

Pemberton regions of the south-west (Figure 1.1) (Rural Industries Research and Development Corporation (RIRDC), 2014).

Figure 1.1 Location of the Manjimup and Pemberton truffle regions in Western Australia. Retrieved from www.winterdownsouth.com/margaret-river-region [28 February 2019]

Between 2007 and 2012, black truffle production grew from 250 hectares to approximately 600 hectares Australia wide, with an estimated 200 to 300 established truffières located throughout New South Wales, northern Tasmania, the highlands of Victoria and Manjimup in Western Australia (AgriFutures Australia, 2017). In 2012 alone, 4,480kg of black truffles were produced within Australia, with an estimated gross value of $5.2 million and by 2017, annual production had increased to 13,000kg (Rural Industries Research and Development Corporation (RIRDC), 2014). Today, truffle production within Australia is a rapidly growing multi-million-dollar industry, retailing at $2,500 to $2,800 per kilogram in 2019 (AgriFutures Australia, 2017; Rural Industries Research and Development Corporation (RIRDC), 2014). As the world's fourth largest black truffle producer, it is estimated that annual production within Australia will exceed 20,000kg by 2020 (McAuliffe, 2019; Rural Industries Research and Development Corporation (RIRDC), 2014).

1. 2 Problem Statement

As discussed above, despite its vital economic value to both regional and local economies and development; knowledge surrounding truffle cultivation is not greatly understood. As the black truffle is an ectomycorrhizal (ECM) fungus, saplings inoculated with truffle spores are required to grow in symbiosis with a host plant (Bradshaw, 2005; Reyna & Garcia-Barreda, 2014). Historical trends show that within a single truffière, certain trees produce more of the black truffle than others (known within the industry as "hot trees") and these trees can change from season to season. Currently, there is little scientific understanding as to what conditions are conducive to a tree becoming "hot" and producing a high truffle yield. This research aims to undercover this information to maintain the economic benefits of truffle production to Western Australia.

1.2.1 Truffle Yield

Within this study, the term *truffle yield* is indicative of the **number** of truffles produced within the study area. The higher the number of truffles produced throughout a harvest season, the higher the estimated economic return for the truffière is. Within this study, truffle **weight** is not used to indicate yield. The longer a truffle is left to develop within the soil, the bigger (heavier) it grows over the harvest season. These larger truffles often begin to develop rot in their centre, just as the outside is developing its aroma and is ready to be harvested. Larger truffles therefore must often be divided into sections, to remove this rot, before selling, decreasing their value. Weight is therefore not a perfect representation of economic return to the truffière. As truffle production is highly dependent upon annual weather conditions, market **value** is often hard to predict and differs from season to season. Prices also differ in response to supply and demand, between regions and proximity to national holidays, such as Christmas (Hall et al., 2007). Even with reliable data, establishing average productivity values is a lengthy process and measuring trends would require decades of statistics (Hall et al., 2007). Market value is also therefore not a viable indicator of yield.

1. 3 Research Objectives

The goal of this research project is to uncover if edaphic factors, such as soil properties, can stimulate truffle production or whether spatial variability in truffle yield is random and therefore due to individual tree performance. The four key objectives of this research include to:

(a) Describe the macro- and micro- scale spatial variability in truffle yield.
(b) Explore appropriate spatial methods used to describe variability across a study area (e.g. Hot Spot Analysis).
(c) Understand whether the distribution of truffle yield across the chosen study area is random.
(d) Determine whether factors and conditions (such as soil properties) could potentially stimulate truffle production.

Additionally, this research will explore the potential management techniques that could be implemented within a truffière to optimise truffle yield. These techniques will emphasise improving the utilisation of resources and inputs to increase potential harvest from the trees within the study truffière. These research aims will assist in sustaining a high level of production within the truffière, so it can maintain its economic benefits to the surrounding south-west region. Any findings resulting from this research could be further be examined to see (if any) relevance exists to other areas within the agricultural sector of Western Australia, especially in the south-west.

1. 4 Organisation of Thesis

This thesis is organised into 5 main chapters: (1) Introduction, (2) Background & Literature Review, (3) Methodology: Data Capture and Collection, (4) Results & Analysis and (5) Conclusion & Recommendations. The first chapter (Introduction) presents the research topic, provides a brief background to the Australian Truffle Industry, and outlines the research significance, objectives and the thesis structure. Chapter 2: Background & Literature Review, familiarises the reader with the basic background knowledge vital to the production of the black truffle, such as required soil and climate conditions, host plants, how a truffière is set up for production and the annual life cycle of the black truffle. This chapter also introduces and describes the study area, including an explanation of why this site is appropriate for this research, as well as outlining what limited studies have previously been conducted in this research field. Chapter 3: Methodology: Data Capture and Collection, is divided into three key parts; Firstly, the required data for this research, secondly, the comparison of various methods tested within this research in order to obtain locational information, as well as the exploration into various spatial methods used to describe variability across a study area. Thirdly, this chapter outlines how the required data for this research was obtained. Chapter 4: Results & Analysis, presents the findings and analysis of this research. Chapter 5: Conclusion & Recommendations, concludes the thesis by restating the findings from this research, along with their corresponding research objective. This chapter concludes by providing some recommendations to improve both the utilisation of resources and potentially increase harvest within the truffière, in order to maintain its economic benefits to both the study truffière and the wider south-west region.

2 BACKGROUND & LITERATURE REVIEW

As outlined in Chapter 1, despite its economic value to both regional and local economies and development, the WA truffle industry is in its infancy, and much of the existing knowledge is focussed on the establishment of truffle plantations (truffières). The industry has grown rapidly in both planted area and yield as the truffières have matured. Historical trends show that within a single truffière, certain trees (known as "hot" trees) produce more of the black truffle than others, and these trees can change from season to season. Industry development now turns to research to assist in understanding what conditions are conducive to a tree becoming "hot" and producing a high truffle yield.

2. 1 Previous Work

Most of the literature available on the *Tuber melanosporum,* like some of those examined in the following sections of this chapter, outlines what conditions have historically been important to the production of the black truffle within the northern hemisphere. Other texts, such as Hall, Brown and Zambonelli's (2007) book, *Taming the Truffle: The History, Lore and Science of the Ultimate Mushroom,* however, have analysed and adapted this historical knowledge to be more applicable for production of truffles in the southern hemisphere. This text is largely referenced within this research as it provides a good understanding of the basics of truffle farming, as well the history of truffle production and reasonings as to why it is such a highly valued commodity worldwide. It further outlines key background information including how to establish a truffière for the production of the black truffle, the conditions conducive to truffle development and growth (such as tree variety) and suitable climatic and soil environments. These suitable soil and climatic conditions are also reconfirmed in other literature such as Bradshaw (2005), Lee (2008) and Mathews & Mitchell (2018).

It should be noted that similar research projects looking to uncover the relationship between soil properties truffle yield for the black truffle, have been conducted in the northern hemisphere. Castrignanò, Goovaerts, Lulli and

Bragato's (2000) paper entitled *"A geostatistical approach to estimate probability of occurrence of Tuber Melanosporum in relation to some soil properties"* hypothesised that "*a soft and well-aerated soil environment might be an essential condition for the growth and production of T. melanosporum*" is an example of one of these studies (Castrignanò, Goovaerts, Lulli, & Bragato, 2000). Using an experimental study area of the University of Perugia (Italy) with similar soil and climatic conditions to those outlined below (see section 2.3 Conditions for Truffle Production) and geostatistical analysis techniques such as Factorial Kriging Analysis [FKA], this study found that areas of well aerated soils were corelated with areas in which the *T. melanosporum* were present (Castrignanò et al., 2000). They noted that further research was required to confirm this relationship. While this study provides a solid recommendation as to what soil properties should be investigated and which analysis tools could potentially be used to explore spatial variability in truffle yield, it is unclear whether these findings carry over to a southern hemisphere location. Additionally, this study would prove more valuable if the number of truffles (rather than just the presence of the black truffle) was measured, hence the importance of this research.

2. 2 History of Truffle Production

While they have been harvested in the wild since the early Renaissance period, commercial production of the Périgord black truffle (*Tuber melanosporum*) did not commence until the early 1800s, when French farmer, Joseph Talon, discovered a rough, yet successful method for cultivation of this highly valued commodity (Zambonelli, Iotti, & Hall, 2015). His technique involved collecting acorns found under oak trees already producing the black truffle and replanting them on small patches of rocky land on his Saint Saturnin lès Apt estate, at the base of the Vaucluse Mountains (Hall et al., 2007; Renowden, 2005; Zambonelli et al., 2015). Less than a decade later, Talon discovered a large crop of black truffles growing beneath some young oak trees located in the same site as the previously planted acorns. Understanding the symbiotic relationship between these two crops, Talon proceeded to purchase cheap stony patches of land adjacent to his estate, sowing acorns into the soils and selling the baskets of resulting truffles at the local market. As neighbouring farmers discovered Talon's technique, the first commercial Périgord black truffle region was created by the late 19th century, and expands throughout areas of France, Italy and Spain (Hall et al., 2007; Renowden, 2005; Zambonelli et al., 2015).

By the 1970's French and Italian scientists had created a method to inoculate the roots of oak and hazelnut trees, grown in controlled greenhouse conditions, with truffle spores for commercial planting in truffières (Rural Industries Research and Development Corporation (RIRDC), 2014; Zambonelli, Piattoni, Iotti, & Hall, 2010). This modern method increased both the availability and accessibility of inoculated trees, spreading the production of the black truffles worldwide, including the United States, Canada, Morocco, Chile, South Africa, Australia and New Zealand (Zambonelli et al., 2015). Today, the black truffle is the most popular variety of truffle available and can collect a high price at market, retailing at approximately $600 to $2,500 AUD per kg (AgriFutures Australia, 2017; Rural Industries Research and Development Corporation (RIRDC), 2014). This is largely due to its difficulty in cultivation, aromatic quality, and flavour. Additionally, the black truffle has the ability to be cooked at low temperatures and incorporated into more recipes than its Italian white truffle (*Tuber magnatum*) counterpart. Today, the *Tuber melanosporum* is largely produced in the southern hemisphere, especially in June to September when, due to climatic conditions, northern hemisphere producers are unable to meet demand.

2. 3 Conditions for Truffle Production

2.3.1 Climate Conditions

The *Tuber melanosporum* traditionally sprouts in various climatic zones through Europe, providing the area is between 40°N and 47°N, and is between 100m to 1000m above sea level (Hall et al., 2007). Around the world today, the black truffle has been produced in many different countries that have similar Mediterranean climates, including regions throughout New Zealand and Australia (Mathews & Mitchell, 2018). Warm summers, and mild to cold winters, like those found in south-west Western Australia, specifically the Manjimup region (34.5°S), provide ideal conditions for truffle production (Lee, 2008). Warm summer temperatures are required to initiate truffle formation, while the cooler winter temperatures trigger final maturation and develop the distinctive aromas of the fungus (Mathews & Mitchell, 2018). Truffles require year-round maintenance of root-zone moisture to facilitate high levels of production. When rainfall does not exceed 1000mm annually, or is particularly low in summer months, irrigation is required to supplement moisture levels (Bradshaw, 2005).

2.3.2 Soil Conditions

For the highest yield of truffles, soil with high pH and abundant calcium are vital (Hall et al., 2007; Mathews & Mitchell, 2018). As outlined in Hall, Brown & Zambonelli (2007), the ideal pH for the black truffle is between 7.2 and 8.3, with 7.9 as the optimal pH level. Additionally, soils require good aeration (frequently referred to as a 'fluffy' structure) to allow the developing truffles to grow (Castrignanò et al., 2000). Soils with decent drainage are also essential to reduce the chance of host plants (such as Hazel and Oak) developing root rot (AgriFutures Australia, 2017). These conditions best correlate to gravelly sandy loam to sandy clay loam surface soils (Figure 2.1).

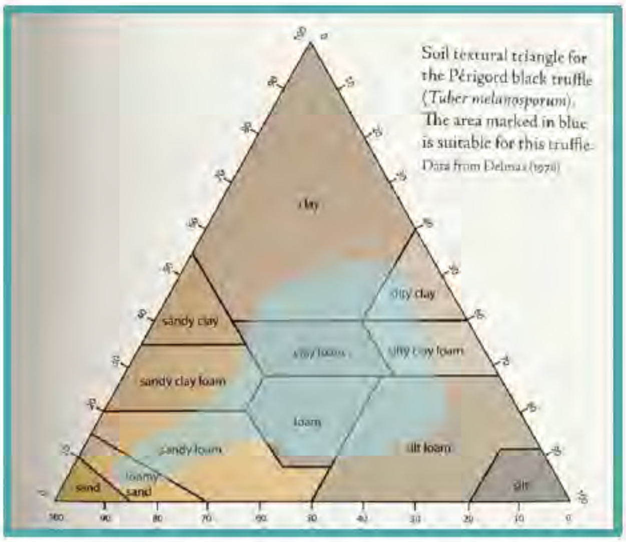

Figure 2.1 Soil texture triangle for the Périgord black truffle. Image adapted from Hall, Brown, and Zambonelli (2007, p. 105)

The black truffle ideally grows in soils with relatively balanced ratio of soil nutrients such as "*phosphorus, calcium, magnesium, nitrogen, potassium, sulphur and trace elements of boron, copper, iron, manganese and zinc*" (Hall et al., 2007). The most productive truffières require high concentrations of plant available calcium and magnesium, as well as moderate levels of phosphorus and little concentration of sodium. Research shows that truffières are rarely productive in soils with an unbalanced ratio of phosphorus to calcium and potassium (Hall et al., 2007). Soils with an excess of calcium are often left with a higher pH than those ideal for the cultivation of the black truffle and can affect the uptake of trace elements. Although it is understood that both soil properties (e.g. temperature, moisture, aeration, trace elements and nutrients) and climate conditions are vital to the production of the black truffle, it is currently unclear which factor(s) have the highest influence over truffle yield (Hall et al., 2007).

2.3.3 Host Plants

As the *Tuber melanosporum* is an ectomycorrhizal (ECM) fungus, saplings inoculated with truffle spores are required to grow in symbiosis with a host plant (Bradshaw, 2005; Reyna & Garcia-Barreda, 2014). Several varieties of both Oak and Hazel are commonly chosen as host plants based on their ability to support this symbiotic relationship, as well as various factors such as tree cover, growth rate and root system (Hall et al., 2007; Rural Industries Research and Development Corporation (RIRDC), 2014). The Common Hazelnut (*Corylus avellana*) is a medium size tree often chosen in the production of the black truffle as it can be planted in a relatively high density (Mathews & Mitchell, 2018). It has a rapid growth rate and a well-developed root system that can produce truffles both earlier and longer than other plant species (Hall et al., 2007). However, as constant shade can affect the formation and development of the black truffle, frequent pruning or herbicides are required to limit the Common Hazelnut's tree cover.

Limitations of host plants are often counteracted in truffières (truffle orchards) by planting more than 1 species of tree. Oak varieties such as the Holm Oak (*Quercus ilex*) and the English Oak (*Quercus robur*) have significantly slower growth rates than Hazels and require more space to grow (Mathews & Mitchell, 2018; Trufficulture Pty Ltd., n.d.). They do however provide extensive root systems, increasing the ratio of truffle to tree, are resilient to adverse cold conditions and require less pruning and overall maintenance (Hall et al., 2007). By planting two or more species, truffières can also protect their truffles against diseases. Pathogens such as *Phytophthora kernoviae*, or sudden oak death, only effects certain Oak varieties. By planting a variety of Oaks and Hazels, truffières can ensure that their orchard can maintain some level of production in cases where a pest or disease is introduced. Additionally, various species of Hazel and Oak can provide secondary sources of income (such as hazelnuts, timber, and firewood) at the end of the productive life of the truffière.

2.3.4 Setting Up a Truffière for Production of the Black Truffle

The Périgord black truffle is a highly valued commodity worldwide, due to the difficulty involved in its cultivation. Establishing a truffière is a lengthy process, and production often doesn't begin until a decade after planting of the host trees. Firstly, the soils on which the trees will be planted must be prepared to the ideal conditions. This includes deep-tilling and liming the soil to loosen the soils and achieve an optimal pH level of 7.9 (Hall et al., 2007). This alkaline reaction takes place over 3-6 months, after which a 4-week delay is required to remove weeds and install necessary irrigation before planting (Trufficulture Pty Ltd., n.d.).

The Oak and Hazel trees, inoculated with truffles spores are then planted in a grid pattern across the prepared site, taking care to alternate the tree type both within and between each row (Figure 2.2) (Mathews & Mitchell, 2018). Over the next 7 to 10 years the roots of the trees develop and colonise the soil, with the first truffles ready to be picked at the end of this period (Hall et al., 2007). With yearly maintenance, truffle yield will gradually increase in the following 10 to 20 years, before reaching maximum yield at the 20-year mark. While still producing truffles, yield from these trees will begin to decline over the 20 to 25-year mark. After this period, the trees should be removed and sold as a secondary source of income in the form of hazelnuts, timber and firewood at the end of their productive life. This production cycle should be alternated across various sites within the truffière to maintain productivity. This process is shown below (Figure 2.3).

Figure 2.2 Oak and Hazel varieties planted in a grid pattern within a truffière. Image taken by author [28 June 2019]

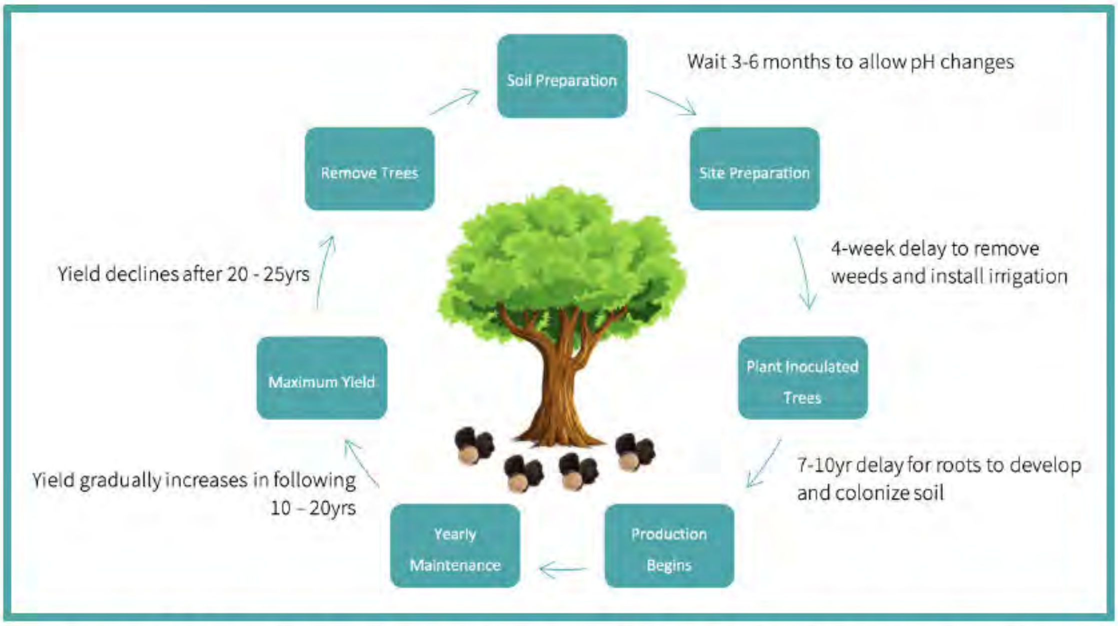

Figure 2.3 Lifecycle of a truffière. Data from Hall et al. (2007); Trufficulture Pty Ltd. (n.d.)

2.3.5 Annual Cycle of the Black Truffle

As outlined by Stahle and Ward (1996), the *Tuber melanosporum* runs on an annual growth cycle that differs between the northern and southern hemisphere. Within the southern hemisphere, in countries such Australia and New Zealand, the annual truffle cycle begins in October when the truffle spores (located 10 to 30cm below the soil, on the roots of the host plants), germinate and activate the truffle fungus (mycorrhizas). Through November, the vegetative part of a fungus, known as the mycelium, spreads through and colonises the soil. During the warmer summer months (December, January and February) the truffle reproduces, forms and begins to grow, while the cooler autumn months (March, April and May) trigger final maturation and develop the distinctive aromas of the fungus (Mathews & Mitchell, 2018). Ripe truffles are then harvested through June, July and August. Any truffles that failed to ripen during the harvest period are left in the ground. In the following month (September) these truffles release new spores back into the soil, which are ready to germinate by October, restarting the growth cycle (Figure 2.4).

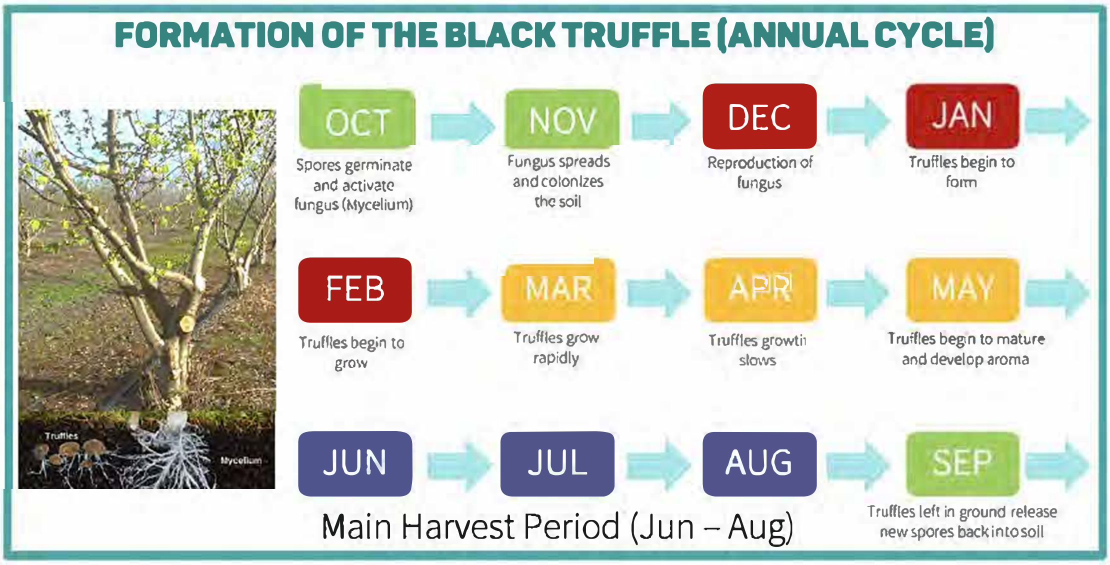

Figure 2.4 Formation of the black truffle in the southern hemisphere. Data from Hall et al. 2007; Stahle & Ward, (1996). Images from Francuski (n.d.); Truffle and Wine Company (n.d.)

2. 4 Study Site – Western Australian Truffière

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2.4.1 History of the Truffière

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2.4.2 Site & Climate Conditions

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2.4.3 Soil Conditions

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2.4.4 Study Area (Site B)

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2. 5 Chapter Summary

Despite its economic value, the WA truffle industry is still in the early stage of production. In recent years, the industry has grown rapidly in both planted area and yield as the truffières have matured. Industry development has turned to research to assist in understanding what conditions are conducive to a tree becoming "hot" and producing a high truffle yield. This chapter outlined the previous studies associated with the production of the black truffle and aimed to familiarises the reader with the basic background knowledge vital to this research.

Historical trends show that within a single truffière, certain trees (known as "hot" trees) produce more of the black truffle than others, and these trees can change from season to season. A review of literature found that previous studies such as Castrignanò, Goovaerts, Lulli and Bragato (2000), are often northern hemisphere centric and fail to link any spatial variability in truffle harvest to specific soil or climatic qualities. Others, such as Hall, Brown and Zambonelli's (2007) however, have analysed and adapted this historical knowledge to be more applicable for production of truffles in the southern hemisphere. This source, backed up by others such as Bradshaw (2005), Lee (2008) and Mathews & Mitchell (2018), outline the specific soil and climate conditions conducive for the production of the black truffle. For a high yield it is expected that a truffière must have:

(a) Warm summers, and mild to cold winters;
(b) Year-round rainfall that exceeds 1000mm annually (or available irrigation);
(c) Soils with A pH level between 7.2 and 8.3, a loose structure and a specific nutrient ratio, and;
(d) A variety of host plants such as Oak and Hazels.

These conditions are explained in more detail throughout the middle sections of this chapter above.

This chapter concluded by introducing the study truffière, including its history and site conditions that are favourable to the black truffle production. It further outlined the specific study area (Site B) and its suitability for this research, due to the observable difference in truffle yield already existing across the site. Although it is understood that both soil properties and climate conditions are vital to the production of the black truffle, it is currently unclear which factor(s) have the highest influence over truffle yield. This research aims to uncover if edaphic factors, such as soil properties, can stimulate truffle production or whether spatial variability in truffle yield is random and therefore due to individual tree performance. The methodology behind this research, as well are the data required to complete the research objectives is outlined in Chapter 3.

3 METHODOLOGY: DATA CAPTURE & COLLECTION

3. 1 Required Data for Research

Before this research was undertaken, little information regarding the study area, including site soil properties, was known. The aim of this research project is to uncover if edaphic factors, such as soil properties, can stimulate truffle production or whether spatial variability in truffle yield (i.e., the number of harvested truffles) is random and therefore due to individual tree performance. Our objectives include determining which soil properties (if any) potentially stimulate truffle production. In order to undertake this research, two key datasets were required; Firstly, the geographical location of each harvested truffle and secondly, a soil profile for the Site B study area.

3.1.1 Required Locational Data

To undertake this research, the location of each harvested truffle needed be obtained. Along with the geographical location (latitude / longitude); the size and harvest time (day/month/year) must also be collected and recorded against each, and every truffle picked in Site B over the harvest season. At our study truffière, picked truffles are sized according to the following scale:

(a) Small – *when the truffle is smaller than an average golf ball (~4cm);*
(b) Medium - *(4cm – 6.5cm);*
(c) Large – *when the truffle is larger than an average tennis ball (~6.5cm).*

Additionally, a descriptive profile of the study area, including the boundaries of the land management units, and the location of all major landmarks including trees, roads, rows, and infrastructure is required.

3.1.2 Required Soil Data

As discussed in Chapter 2, in order to produce the *Tuber melanosporum*, specific soil qualities are required. Ideally, for a high production of the black truffle, the soils must have:

(a) A pH level between 7.2 and 8.3 (7.9pH is ideal);
(b) A loose structure (soils with high gravel content, e.g. gravelly sandy loam to sandy clay loam are ideal); and,
(c) A specific nutrient ratio.

As outlined in Hall (2007), truffle production requires a "*relatively balanced ratio of soil nutrients such as "phosphorus, calcium, magnesium, nitrogen, potassium, sulphur and trace elements of boron, copper, iron, manganese and zinc*" (Hall et al., 2007). Soils also require high concentrations of plant available calcium and magnesium, as well as moderate levels of phosphorus and little concentration of sodium. Research shows that truffières are rarely productive when they contain soils with an unbalanced ratio of phosphorus to calcium and potassium. Soils with an excess of calcium are often left with a higher pH which can affect the uptake of trace elements (Hall et al., 2007).

In order to determine why variability in truffle yield occurs across a study area, soil information, such as soil structure, quality, nutrients, moisture content and drainage, as well as tree information, such as tree diameter[1] and root depth, will also be required. To obtain this information, a soil sampling method must be created. This method must ensure that any collected soil data is representative of the entire study area. By creating a soil profile for the study area, areas of high truffle yield can be compared to specific soil qualities, and correlations can be made between soil properties and truffle yield.

[1] Tree height is not considered, as diameter is a better indication of biomass (productivity) (Shaw, Lankey, & Jourdan, 1996)

3. 2 Testing of Various Methods used to Obtain Location Information

Within this research, two different methods were tested to obtain the required locational data. Firstly, a Global Position Systems (GPS) set-up using a relative positioning technique, and secondly, mobile data acquisition, such as the Environmental Systems Research Institute (ESRI)'s *Collector for ArcGIS* application.

3.2.1 Required Accuracy

As stated in Chapter 2, within the Site B study area, Oak and Hazel trees inoculated with spores of the black truffle are planted every 5m along the 106 rows within the site. Any method used to obtain locational data must therefore be able to meet the following requirements:

(a) it must be able to record geographical location of each harvested truffle at a relatively high speed;
(b) be accurate to less than 5m (the distance between the trees within each row), and;
(c) be available offline due to the isolated location of the study area.

As accuracy is defined as the degree of closeness or conformity of an observation to its "true value", the positioning technique can be deemed accurate if the horizontal positions of the harvested truffles are taken within ±5m (95% confidence) to their true position (M. Kuhn, 2018a). This accuracy level was chosen as it allows a clear link to be made between a harvested truffle, and the corresponding Oak or Hazel tree it developed from, without compromising the cost and time limitations of the project.

3.2.2 Global Positioning Systems

As discussed above, any method chosen to obtain locational data within this research must be accurate to 5m (95% confidence). Global Positioning Systems (GPS) using a differential (or relative) positioning technique may be used to map the study area, and the location of each harvested truffle, with a relatively high (cm) accuracy level with 95% confidence (El-Rabbany, 2006). An example of this set up is a Real Time Kinematic (RTK) approach, in which two GPS receivers (carrier-phased based) simultaneously tracking four or more common satellites, can obtain a locational accuracy down to the cm level (El-Rabbany, 2006; M. Kuhn, 2018b). Within an RTK survey, one receiver (known as the base station) is stationary and is located at a "known" position and the second receiver (known as the rover station) is then used to mark observed locations (such as that of a harvested truffle). A RTK approach is ideal when a survey involves a large number of unknown points (such as truffles), when the coordinates of the unknown points are required in real time, and the line of sight between the base and the rover is relatively unobstructed (El-Rabbany, 2006; M. Kuhn, 2018b). An RTK system uses a radio link to transmit observations and correction positions between the base and the rover, in order to gain precise locational information. An example of an RTK set up is outlined below (Figure 3.1).

3.2.3 Mobile Data Acquisition

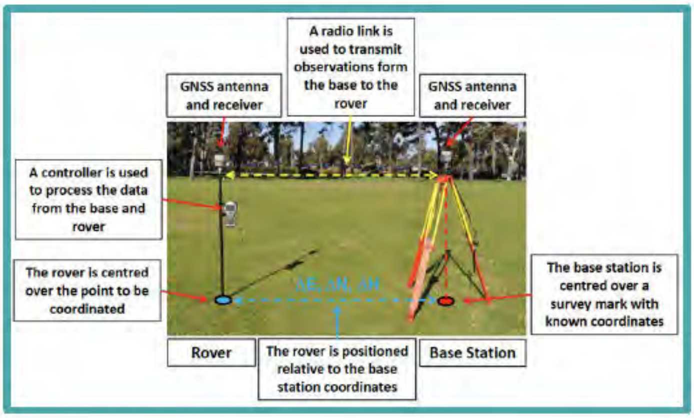

Figure 3.1 RTK positioning technique used to obtain coordinates at a cm-level accuracy. Figure obtained from M. Kuhn (2018c)

Mobile mapping applications, such as ESRI's *Collector for ArcGIS* app, are a secondary method to collect locational information at the study truffière. For an in-field application to be suitable for this research, it must be meet the following requirements:

(a) it must be able to record geographical location of each harvested truffle at a relatively high speed;

(b) be accurate to less than 5m (the distance between the trees within each row);
(c) be relatively low cost, and;
(d) be available offline (able to collect data even if Wi-Fi signal is not available), due to the isolated location of the study area.

Additionally, the app must be able to obtain the geographical location (latitude / longitude), the size (small, medium, large) and the harvest time (pick date and time) of each truffle harvested during the season. For in field use, the app should also ideally be user friendly (i.e., lightweight for all day use) and quick to "pick up" (record) the data. Ideally, any data collected from the app, should be able to be loaded into software for further spatial analysis.

3.2.3.1 Collector for ArcGIS

First launched in the early 2010s, Collector is a mobile mapping app that meets all the requirements set by this research. Today, the updated *Collector for ArcGIS* app allows you to gather both spatial (location) and non-spatial (size, time and date) data for features, and allows users to begin to recognise and interpret spatial patterns in the field (Pánek & Glass, 2018; Pundt & Brinkkötter-Runde, 1998). Images of each harvested truffle can also be recorded within the app for later reference. Additionally, Collector can "…*capture and edit assets and observations in field that seamlessly integrate into ArcGIS*" and any data gathered by the app is "*automatically uploaded to ArcGIS online when service is available… and can be temporarily stored on the device when areas lack mobile connectivity*" (Environmental Systems Research Institute (ESRI), 2020; John Hopkins Sheridan Libraries, 2017).

The collector app can be downloaded onto all major mobile devices (iOS, Windows and Android), and is therefore lightweight and easy to use in the field. In terms of accuracy, the app uses the GPS receiver integrated into the handheld phone to determine geographical location. When using the iPhone 11 Pro, geographic location is determined through the use of the following location specifications: the integrated GPS/ GNSS receiver, digital compass, Wi-Fi or mobile data, and the iBeacon microlocation. This tool results in a ~5m horizontal accuracy level (95% confidence) (Figure 3.2). To meet the accuracy requirements for this study, this level can be increased with the use of an additional GPS receiver, connected via Bluetooth to the handheld device. Examples of external receivers, as well as their capabilities are outlined in Appendix B.

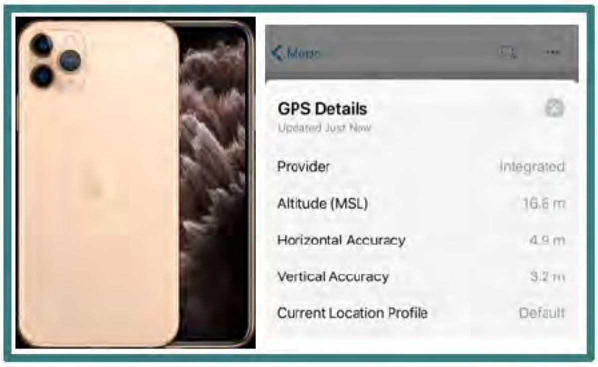

Figure 3.2 Collector accuracy using the iPhone 11 Pro. Image from https://www.apple.com/au/shop/buy-iphone/iphone-11-pro [screenshot taken 3 January 2020]

3.2.3.2 Preparing for Data Collection

As the Collector app only has the ability to obtain vector data (points, lines and polygons), within this research project, *Collector for ArcGIS* will ideally be used to obtain points, each of which will represent a truffle harvested at the study truffière. Along with the geographical location of each truffle, the harvest time and size (small, medium or large) of the truffle will be recorded against each point. Additionally, for future reference a photo is attached to the point. However, before field mapping can take place, preparation of the survey must be undertaken. To prepare a survey within Collector, a subscription to an ArcGIS organisation account is required.

A survey is prepared by opening ArcCatalog and creating a new Geodatabase. Within this database, a new point feature class, entitled "harvestedtruffles" is created, using GDA2020 MGA Zone 50 (or the corresponding zone for the truffière) as the coordinate system. Three fields should be added to this feature class, including the (1) Harvest Time (data type: Date), (2) Size (data type: long integer) and (3) Picture (data type: text, 1000 max characters). In order to automatically add the date and time to each feature when a point is created, the **Harvest Time** field should be selected in the "Create Date Field" under the Editor Tracking tab (found in the feature class properties menu). A domain should also be added to the **Size** field, to allow the user to select from the pre-coded options (small, medium or large) when collecting data in the field.

After preparing the survey, the "harvestedtruffles" feature class should be added to a new map in ArcMap (desktop). To allow the user to attach an image to each point within the field, the *attachments* function should be added to the truffle feature class. The map should then be saved and shared as a service on ArcGIS online, using the ArcGIS Organisation account as the hosted service connection, ensuring all operations are allowed. This "harvestedtruffles" hosted feature layer should then be opened in a new map on ArcGIS online. To allow data to be collected in the field, this map should be shared to the organisation as a web map. When the web map is made available to the public, anyone with a link to the web map will be able to add points to the feature layer through the use of the *Collector for ArcGIS* app. When kept private, the link and a personal user account is required. Supporting images for the process outlined above are available in Appendix C.

3.2.3.3 Collecting Locational Data in the Field

The process for using the *Collector for ArcGIS* to obtain in field locational data is shown below (Figure 3.3). Firstly, the shared Web Map, created in the above section, should be opened in the Collector app. Points are added to the feature class using the *add feature* tool, ensuring the corresponding truffle size is selected under the *Size* field, and a photo is attached using the *Take Photo* button. Once added, these points are then automatically uploaded to ArcGIS online. The "harvestedtruffles" feature layer can then be reloaded into ArcGIS (desktop) for further spatial analysis to determine the variability in truffle yield across the study area. Appendix D provides supporting images including (a) an example point obtained using the Collector app, as well as its corresponding data, and (b) the feature layer reloaded into ArcGIS for further spatial analysis.

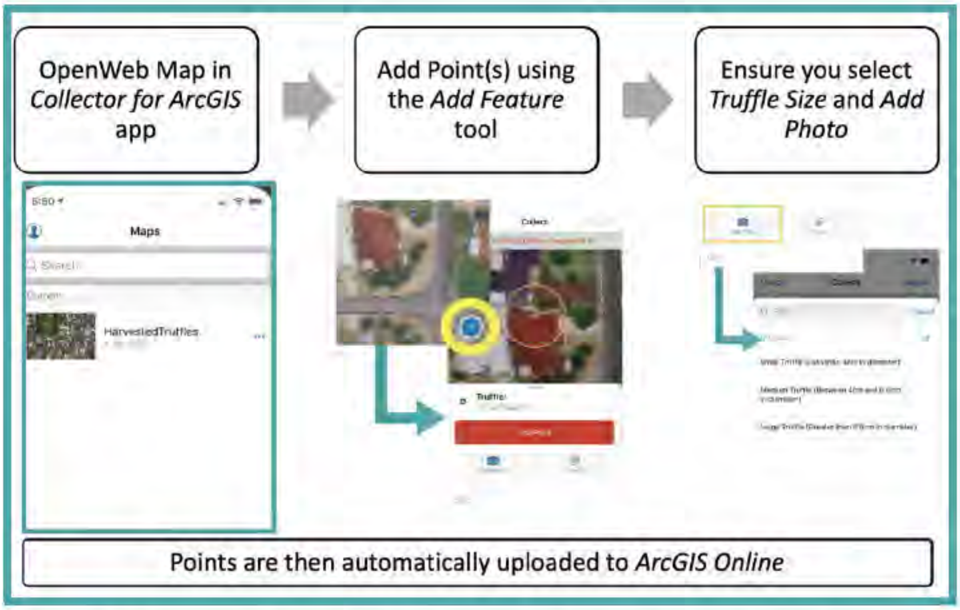

Figure 3.3 Process of using the *Collector for ArcGIS* app to obtain locational information in the field

3.2.4 Chosen Method for Study

Before the commencement of the 2019 harvest season, fieldwork was undertaken at the study truffière, to determine which method to obtain locational data should be utilised in this research project. For either method to be appropriate, the recorded location of each harvested truffles must be within ±5m (95% confidence) to their true position.

Through this fieldwork, it was found that a Global Positioning System using the RTK approach would be inappropriate to obtain the geographic location of each harvested truffle within Site B, as it failed to meet the accuracy requirements. In order to obtain this locational information, measurements had to be taken under significant amounts of tree cover. As GPS receivers obtain geographic location using a process known as *trilateration* (which requires a constant line of sight between the receiver and the satellites in the sky) any obstructions, such as tree cover, can limit the accessibility of these satellites and cause frequent loss of continuity of satellite signals (Bakuła, Oszczak, & Pelc-Mieczkowska, 2009; M

Kuhn, 2018). This reduces the accuracy of the observations (Bakuła et al., 2009). Although this method cannot be used to obtain the geographic location of each harvested truffle within Site B, it is able to obtain a descriptive profile of the study area, in areas where limited or no tree cover is present. This method was therefore used to obtain key information such as the boundaries of the land management units, and the location of all major landmarks including the start and end tree of each tree row.

Whilst fieldwork was undertaken, and through discussion and consultation with the truffière manager, it was revealed that the harvest data was already being recorded at the study truffière. Each picker within Site B is provided with a mobile device, and using a method similar to that outlined in *3.2.3 Mobile Data Acquisition*, obtains the geographic location (within the required accuracy level), size and pick date and time of each harvested truffle within Site B. As this information collected by the pickers in Site B met all of the data requirements, it was decided that the study truffière would provide the required harvest data. This was beneficial as it reduced the cost of the research (as the same data wasn't being collected twice) and meant that the harvest season wasn't slowed down or impeded by having to train the pickers in a different mobile system. To complete this research, the study truffière provided two csv files containing the truffle data for the 2019, 2020 and 2021 harvest seasons. An example of this csv file is shown below (Table 3.1).

Table 3.1 Example data from the 2020 truffle data csv file

PickDateTime	Latitude	Longitude	Size
2020-06-05 14:31:41.000	-34.28411833	116.0561283	Small
2020-06-05 14:35:59.000	-34.28366667	116.0561733	Small
2020-06-09 08:51:24.000	-34.28359167	116.0557817	Small
2020-06-09 08:52:09.000	-34.28359167	116.0557817	Medium
2020-06-09 08:52:11.000	-34.28359167	116.0557817	Small
2020-06-09 12:59:44.000	-34.28412667	116.055955	Medium
2020-06-09 12:59:45.000	-34.28412667	116.055955	Medium
2020-06-09 13:00:40.000	-34.283955	116.0560683	Small
2020-06-05 14:29:58.000	-34.28364167	116.05609	Small

3. 3 Exploration of Various Methods used to Describe Variability

This research aims to examine the spatial variability of truffles and determines how this information can be understood to improve utilisation of resources and management techniques. In order to explore what factors (such as soil properties) could stimulate truffle production, firstly it must be determined whether a clear spatial variation in truffle yield exists in the Site B study area. This variation will be determined using various GIS spatial analysis tools. This section utilises truffle yield data provided by the study truffière for their 2019, 2020 and 2021 harvest seasons, to outline how the *Average Nearest Neighbour Ratio* and *Optimised Hot Spot Analysis* tools can be used to determine whether a clear spatial clustering of truffles within the study area exists. If significant clustering occurs, then further analysis can determine which factors stimulate truffle yield. This research assists in identifying options to optimise the productivity of the truffière, in order to maintain its benefits to the wider south-west region.

3.3.1 Preparing the Data

In order to determine whether spatial variability in truffle yield exists across the study area, ArcGIS Spatial Statistics tools were utilised. Firstly, a personal geodatabase was created in ESRI's ArcGIS software to store and manipulate the yield data. Secondly, the csv file provided by study truffière, containing truffle yield data for the 2019 harvest season (May to August), was exported to this geodatabase using the *Export to Geodatabase (single)* tool. For each harvested truffle, this csv file contained the following parameters (Figure 3.4):

	A	B	C	D
1	PickDateTime	Latitude	Longitude	Size
2	18/6/19 7:10	-34.28834	116.05389	Small
3	18/6/19 7:12	-34.288658	116.0576	Medium
4	18/6/19 7:12	-34.288645	116.05764	Small
5	18/6/19 7:12	-34.288635	116.05764	Medium
6	18/6/19 7:12	-34.288278	116.05391	Small
7	18/6/19 7:12	-34.288378	116.05399	Medium
8	18/6/19 7:12	-34.288657	116.05759	Medium
9	18/6/19 7:12	-34.288652	116.05764	Medium

Table

KY20190618_OVT_Picks

OBJECTID *	PickDateTime	Latitude	Longitude	Size	Shape *
1	18/06/2019 7:10:00 AM	-34.28834	116.05389	Small	Point
2	18/06/2019 7:12:00 AM	-34.288658	116.057598	Medium	Point
3	18/06/2019 7:12:00 AM	-34.288645	116.057635	Small	Point
4	18/06/2019 7:12:00 AM	-34.288635	116.05764	Medium	Point
5	18/06/2019 7:12:00 AM	-34.288278	116.053913	Small	Point
6	18/06/2019 7:12:00 AM	-34.288378	116.053993	Medium	Point
7	18/06/2019 7:12:00 AM	-34.288657	116.057592	Medium	Point
8	18/06/2019 7:12:00 AM	-34.288652	116.057643	Medium	Point
9	18/06/2019 7:12:00 AM	-34.288657	116.057592	Medium	Point
10	18/06/2019 7:12:00 AM	-34.288652	116.057643	Small	Point

Figure 3.4. Truffle yield .csv file (left) converted to feature class in the geodatabase (right)

(a) The date and time the truffle was picked;
(b) It's location in latitude & longitude, and;
(c) It's size (small, medium or large).

Once exported the latitude and longitude were converted to a projected coordinate system (GDA2020 MGA Zone 50) using the *Convert Coordinate Notation* tool. Converting the data to a projected coordinate system was important as it allowed a base map to be placed under the truffle yield data for further data interpretability. This process was repeated for the 2020 and 2021 harvest seasons csv files.

3.3.2 Average Nearest Neighbour Ratio

To determine how the 2019, 2020 and 2021 harvested truffles are distributed (i.e. exhibit a clustered, dispersed or random spatial pattern) across the Site B study area, the *Average Nearest Neighbour (ANN)* tool was used. This tool calculates the nearest neighbour index, or the average distance of a feature (i.e. truffle) to its nearest neighbour (i.e. another truffle) compared to the expected average distance, and is calculated using the following formula (Grekousis, 2020; Pimpler, 2017) :

$$NN = \frac{observed\ mean\ distance}{expected\ mean\ distance} = \frac{d_{min}}{E(d)} = 2\bar{d}_{min}\sqrt{n/a}$$

Where:

$$\bar{d}_{min} = \frac{\sum_{i=1}^{n} d_{\min}(S_i)}{n}$$

$$E(d) = 1 \,/\, \left(2\sqrt{n/a}\right)$$

$\bar{d}_{min}$ is the average nearest neighbours distance of the observed spatial pattern;

$d_{\min}(S_i)$ is the distance of event (truffle location) S_i to its nearest neighbour;

n is the total number of events (i.e. truffles);

$E(d)$ is the expected value for the mean nearest neighbour distance under complete spatial randomness, and;

a is the area of the study region (or if not defined, the minimum enclosing rectangle around all events) (Grekousis, 2020). Within this study this value was not defined to ensure every truffle harvested during its corresponding harvested season was included in the ANN calculation.

This tool produces 3 values:

(a) **The ANN ratio**: returns a value between 0 and 1. If the ANN < 1, then the dataset shows clustering of features, where an ANN > 1 shows dispersion of features within the dataset.

(b) **Z Score**: the standard deviation or confidence level of the results. The Z score and how it corresponds to confidence levels is shown in Table 3.2.

(c) **P Value**: the measure of probability that a random process caused the spatial pattern. Small p values indicate that the spatial variability in the dataset is not due to randomness. P values can also be associated with confidence levels as shown in Table 3.3 (Pimpler, 2017).

Table 3.2 Z Score and its corresponding confidence level

z-Score (Standard Deviation)	Confidence Level
<- 1.65 or > +1.65	90%
< -1.96 or > +1.96	95%
<-2.58 or > +2.58	99%

Table 3.3 P-Value and its corresponding confidence level

P-value (Probability)	Confidence Level
< 0.10	90%
< 0.05	95%
< 0.01	99%

The results of the ANN tool for the 2019, 2020 and 2021 harvest seasons are shown below (Table 3.4). The complete ANN reports for these datasets are available in Appendix E.

Table 3.4 ANN results for the 2019 and 2020 harvest season

Dataset	Observed Distance	Expected Distance	ANN Ratio $\frac{observed\ distance}{expected\ distance}$	Z-score	P-Value	Distribution
2019 Harvest Season	0.5121	1.3525	0.3786	-215.41	0.00	Clustered
2020 Harvest Season	0.3273	0.9964	0.3285	-313.56	0.00	Clustered
2021 Harvest Season	0.4041	1.2813	0.3153	-251.01	0.00	Clustered

The above table demonstrates that the 2019, 2020 and 2021 reports returned ANN ratios less than 1.0. This figure shows that a significant level of clustering is evident within each dataset, with a high confidence level that this clustering is not due to a random process. Within the Site B study area, this is a clear indication of spatial variability, in which harvested truffles are clustered across the site.

3.3.3 Optimised Hot Spot Analysis

In order to map the clustering of the 2019, 2020 and 2021 harvested truffles, the *Optimised Hot Spot Analysis* tool was utilised. This tool was chosen as it uses the location of features, rather than a particular attribute to locate and map locations of statistically significant hot and cold spatial clusters (i.e. spatial variability) within the dataset (Grekousis, 2020; Pimpler, 2017). These clusters are mapped using the Getis-Ord Gi* statistic:

$$G_i(d) = \frac{\sum_j w_{ij}(d)x_j}{\sum_{j=1}^{n} x_j}, j \neq i$$

Where:

d is the estimated range of observed spatial autocorrelation;

$\sum_j w_{ij}(d)$ is the sum of weights for $j \neq i$ within distance d;

n is the total number of observations (i.e. truffles), and;

x_j is the attribute value of feature j.

3.3.3.1 Spatial Resolution

This tool was run multiple times to determine an appropriate spatial resolution (i.e. the size of one cell on the ground) for this study. Spatial resolution does not indicate accuracy, but instead allows for more or less detail to be mapped. For example, a spatial resolution of 10m means one cell on the optimised hot spot is equal to 10m x 10m on the ground. A smaller spatial resolution decreases the cell size and therefore increases the ability to distinguish two spatially adjacent features on the ground, especially in high density areas, but is not necessarily more or less accurate (in terms of location) than a 10m, 5m or 2m spatial resolution (Bakker et al., 2009). When running the *Optimised Hot Spot* tool, all fields were automatically calculated, apart from the cell size (in metres).

To determine an appropriate spatial resolution for this study, the *Optimised Hot Spot* tool was run multiple times on the 2019 harvested truffle shapefile, changing the spatial resolution (cell size) from 10m, to 5m and finally 2m. Figure 3.5 displays how different spatial resolutions affected the optimised hot spot for the 2019 harvest season within Site B (green). As the spatial resolution increases (i.e. the number representing one grid on the ground decreases), more detail within the hot spot is available. When increasing the spatial resolution from 10m (Figure 3.5 right) to 2m (Figure 3.5 left) for the 2019 harvest season *Optimised Hot Spot*, more accurate information about which trees are potentially producing a higher number of truffles is shown due to less generalisation in the data. From this test, a spatial resolution of 5m was chosen as the default spatial resolution to display the truffle data for the 2019, 2020 and 2021 harvest seasons. This spatial resolution was chosen as it correlates to the distance between the trees within Site B and allows for the identification of which trees are producing a higher yield of truffles (hot spots), and which trees are producing a lower yield in truffles (cold spots).

Figure 3.5 Site B: varying levels of detail based on different spatial resolution (2m, 5m and 10m from left to right)

3.3.3.2 Interpreting the Optimised Hot Spot

How the colour of each cell in the *Optimised Hot Spot* relates to the yield of truffles is shown below (Figure 3.6). Within this study, areas of high clustering (i.e. higher truffle yield) are shown in reds and are considered to be a "hot spot". Areas of low clustering (i.e. very low truffle yield) are shown in blue and are considered to be a "cold spot". Clear areas do not exhibit a clustering pattern, and while they may produce truffles, are not statistically significant enough to be considered "hot".

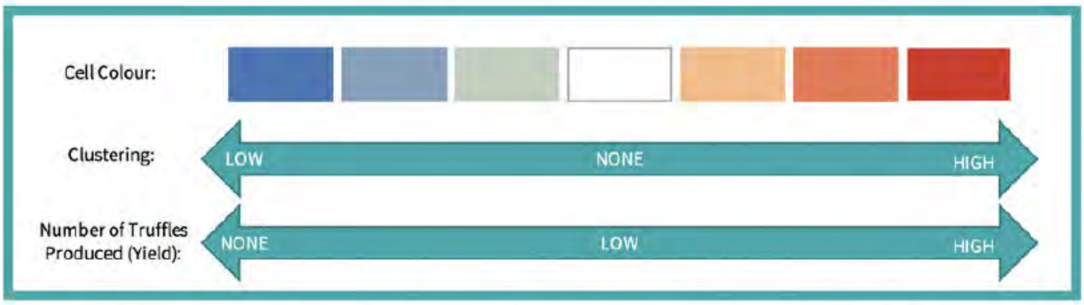

Figure 3.6 How cell colour relates to truffle yield in the Optimised Hot Spot analysis

3.3.3.3 Variation in Truffle Yield - Site B

The *Optimised Hot Spots* for the 2019, 2020 and 2021 harvest seasons are shown below (Figure 3.7 to 3.9). These hot spots demonstrate that spatial variability in truffle yield does exist within the Site B study area. Areas along the south and south-eastern borders of Site B consistently show positive clustering and therefore produce a high yield of truffles over the 2019 and 2020 harvest seasons. Portions of the north-west and north-eastern quadrant of the study area show negative clustering and therefore produced little, if any, truffles over these harvest seasons. In 2019, these cold spots spread from the north-western to the north-eastern corners of Site B but shifted towards a more central north clustering in 2020. Significant positive clustering of harvested truffles was consistently present in the lower south-western corner of the study area. This positive clustering shifted further north-east for the 2020 harvest season.

These hot spots therefore validate the historical trend that certain trees within a single truffière (known as “hot” trees) produce more of the black truffle than others, and these trees can change from season to season. Although the 2021 harvest season showed similar clustering patterns to those exhibited in 2019 and 2020 harvest years, the 2021 harvest data will not be utilised within the analysis section of this thesis. This is due to the underutilisation of the mobile recording devices that pickers used in 2021 to correctly record the truffles harvested within the Site B study area. The underreporting of the 2021 harvest data could potentially skew the analysis, and therefore will not be used in this study. A more detailed analysis of these hot spots produced in the 2019 and 2020 harvest seasons are available in Chapter 4.

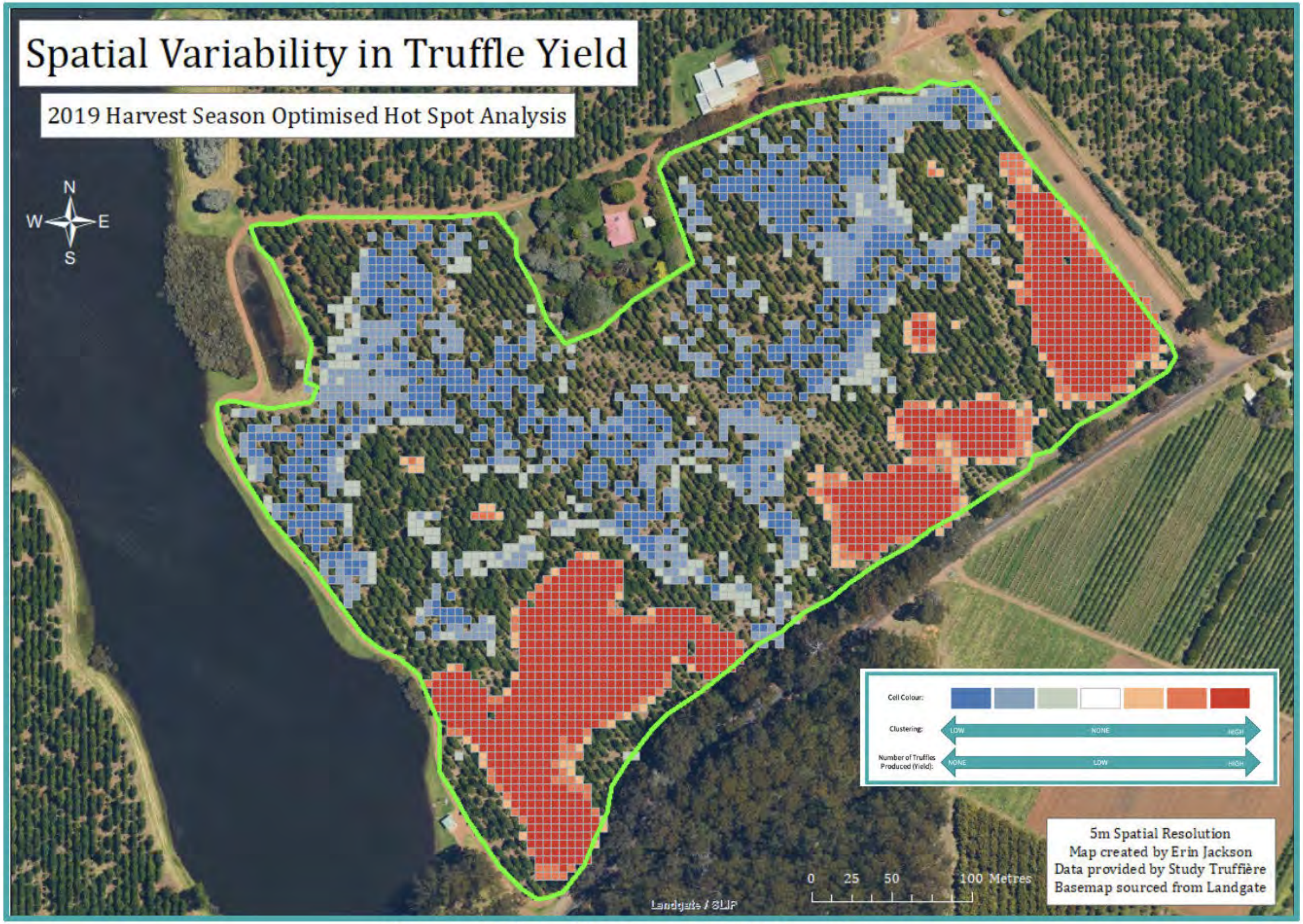

Figure 3.7. Optimised Hot Spot for the 2019 harvest season

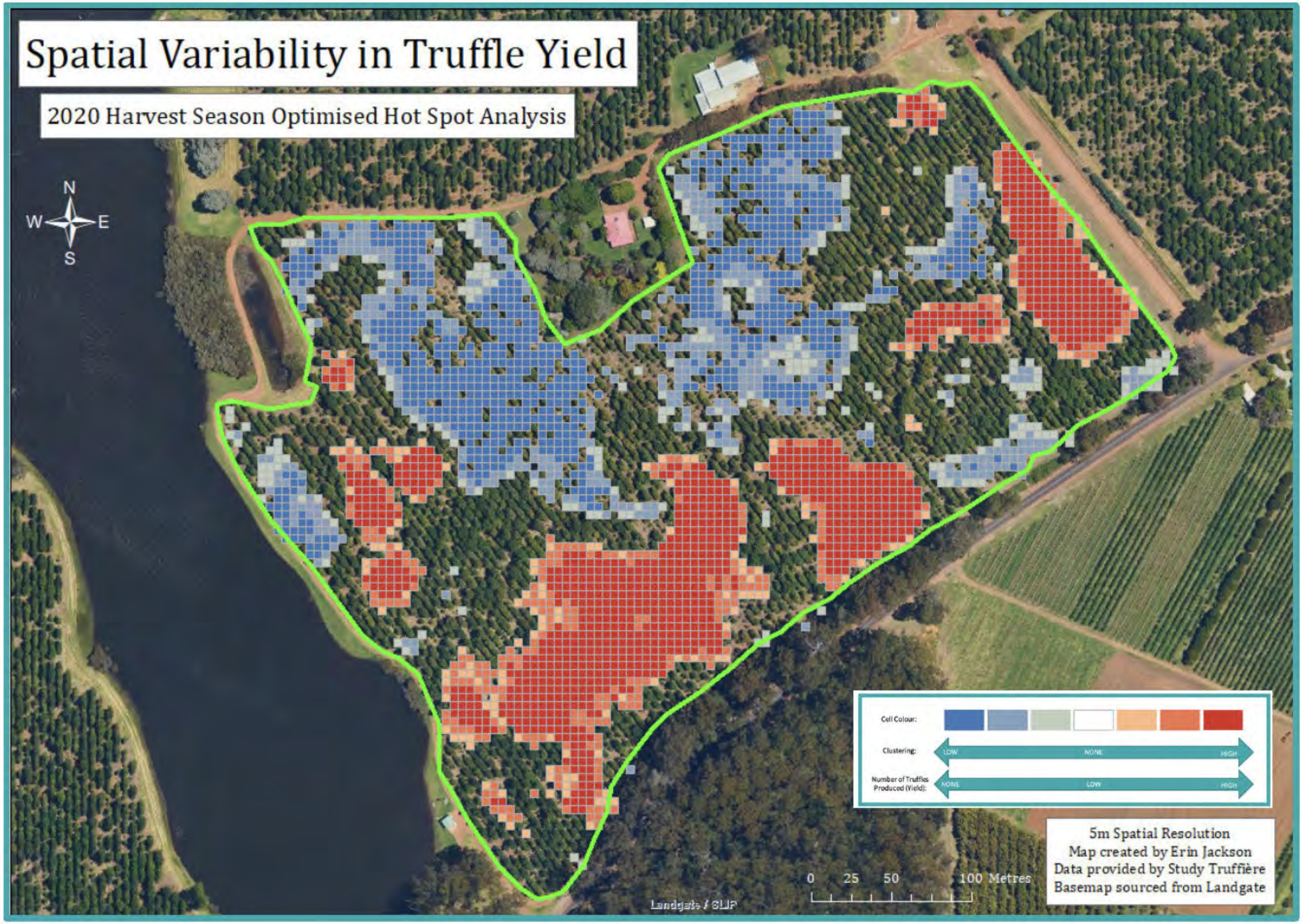

Figure 3.8 Optimised Hot Spot for the 2020 harvest season

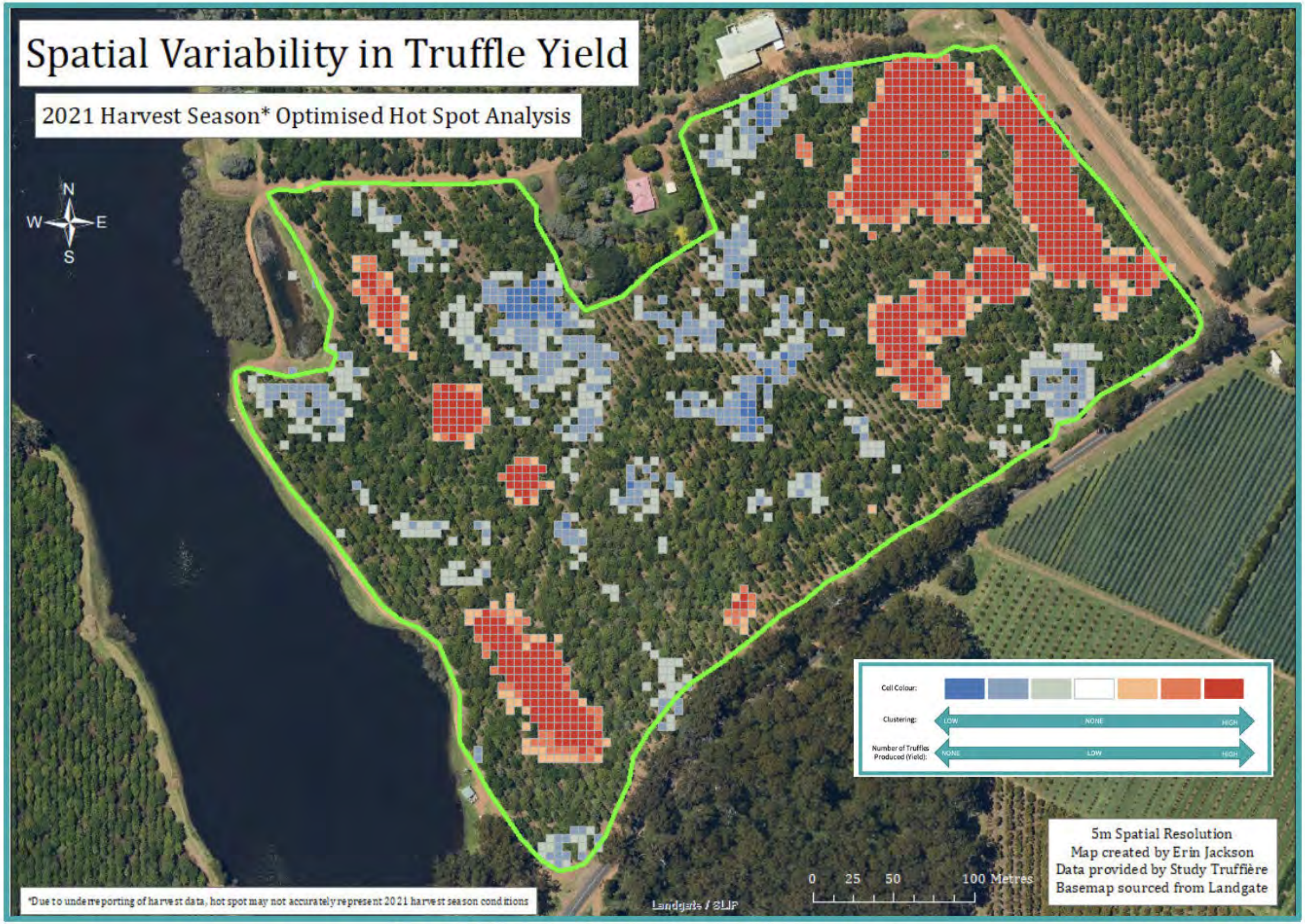

Figure 3.9 Optimised Hot Spot for the 2021 harvest season

3.3.4 Summary of Spatial Variability Methods

This section outlines the processes involved in determining whether a significant spatial variability (i.e. clustering) in truffle yield across the site B study area exists for studied harvest seasons (2019 and 2020). Through the use of *Average Nearest Neighbour* and *Optimised Hot Spot Analysis* ArcGIS tools, it is evident that certain trees within Site B are producing more truffles than others. These trees also change from season to season. In order to explore what factors are potentially stimulating this clustering, information regarding soil temperature, moisture, aeration, trace elements and nutrients across the study site is required. Within this study, this soil profile was obtained through soil sampling and will provide some insights as to what conditions are conducive to a high truffle yield.

3. 4 Methods for Obtaining Required Soil Data

As discussed above, a soil profile for the study area will allow areas of high truffle yield to be compared with specific soil qualities, allowing correlations between soil properties and truffle yield to be made. This soil profile should ideally include information on:

(a) soil structure (measured through particle size);
(b) nutrients (specifically plant available calcium, magnesium, phosphorus, sodium, potassium and nitrogen);
(c) pH, and;
(d) moisture content and drainage.

Additionally tree information, such as tree diameter and root depth, will also help indicate towards health and productivity of the soils.

Before soil surveying can occur in the field, a sampling strategy appropriate for the research must be designed. Sampling is necessary as it would be highly impractical, due to cost and time, to measure every piece of soil throughout the study area (Webster & Lark, 2012). Instead, observations are recorded at a number of selected areas within the field, known as sampling units, and the results are then extrapolated (statistically inferred) to represent the entire study area. Within sampling, there are always some levels of error and uncertainty. Criteria such as variance and bias are therefore used to assess the quality of the sampling strategy. There are two fundamental approaches to soil sampling; the design-based approach, based on traditional sampling theory, and the model-based approach, based on geostatistical sampling (Brus, 2020; Brus & de Gruijter, 1997). These methods differ in the ways in which concepts such as randomness are introduced, and the methods used to select sampling units and for statistical inference (Brus, 2020; de Gruijter, Brus, Bierkens, & Knotters, 2006; Papritz & Webster, 1995a)

3.4.1 Design Based vs. Model Based Sampling

Within a design-based approach, sampling units are selected by probability (random) sampling, and statistical inference is based on the sampling design (i.e. not determined through the use of a model). Examples of random sampling methods include Simple Random Sampling (SI) and Stratified Simple Random Sampling (STSI) (Brus & de Gruijter, 1997; Papritz & Webster, 1995b). As the sampling units are selected at random, the probability of a unit being sampled is known, and this probability provides the basis for statistical inference from the data (Brus, 2020; de Gruijter et al., 2006). The source of randomness within a design-based approach is therefore the random selection of a sampling unit (Viscarra Rossel, Brus, Lobsey, Shi, & McLachlan, 2016). When making inferences (such as calculating the weighted average of the dataset) using a design-based approach, data is weighted using the selection probability rather than their geographical coordinates of the sampling units (de Gruijter et al., 2006). The accuracy of inferred data depends upon the sampling method used.

Within a model-based approach, there are generally no requirements for the selection of sampling units and the statistical inference is based on the model used in the sampling design. Purposive sampling is often favoured over probability sampling, due to its efficiency, and these methods include Centred Grid Sampling, Spatial Coverage Sampling and Geostatistical Sampling (Brus, 2010; de Gruijter et al., 2006). As the model-based approach generally uses purposive sampling, the source of randomness and inference methodology is introduced via the model of spatial variation (Brus, 2010). When making inferences using a model-based approach, data is weighted using a function of the coordinates of the sampling locations, as defined in the chosen model (de Gruijter et al., 2006)

3.4.2 Choosing an Appropriate Sampling Method

Since the 1980s, many soil scientists have shifted their sampling methods from a design-based (DB) to a model-based (MB) approach when conducting soil sampling, due to the belief that the MB approach better represents earth sciences through the use of geostatistics (Brus & de Gruijter, 1997). However, papers such as de Gruijter and ter Braak (1990), clearly state that "*this is a misconception…and both sampling approaches are valid and have their merits*" (Brus, 2020). An appropriate method depends upon both your aims and expected outcomes (or uses) of the soil data. Some rules and guidelines for selecting the appropriate sampling method are outlined in both Brus & de Gruijter (1997) and de Gruijter (2006), and take into account the target variables and parameters, any known prior information, the cost involved, and the efficiency required. Generally speaking, a DB approach is suitable if your interest is in the population mean(s) of a restricted number of subpopulations. While it is not highly efficient, its strengths lie in its validity of estimates. The MB approach is appropriate if your aim is to the map the soil property of interest and want to use this map to predict values as precisely as possible. The MB approach is highly efficient, but only when checks are made to ensure the chosen model is valid (Brus, 2020; Viscarra Rossel et al., 2016). If the required sampling task is dependent upon the validity of the results, it is best to use a design based approach, as no modelling assumptions are made (Brus, 2020).

It should be noted that one does not have to pick between a fully design-based or model-based approach. As outlined by Brus (2020), there are 3 possible approaches within soil sampling; a fully designed based approach, a fully model based approach, and the model-assisted approach. The model-assisted approach is design-based in theory (i.e. the sampling units are selected via probability sampling, and bias and variance are design-based) but utilises different regression coefficients from the model-based approach to try to introduce its strengths, such as the increase in the accuracy of estimates, into the sampling method (Brus, 2020). The properties for these approaches are outlined below (Table 3.5).

Table 3.5 Summary of sampling approaches and their associated properties

Approach	Sampling	Statistical Inference	Source of Randomness	Regression Coefficients	Quality Criteria	Selection Criteria
Design-based	Probability sampling	Design-based (no model used)	Selection of sampling locations	No model	Design-bias, design-variance	interest is in the population mean(s) of a restricted number of subpopulations. Validity over efficiency.
Model-assisted	Probability sampling	Model-assisted	Selection of sampling locations	Population parameters	Design-bias, design-variance	Increased accuracy of estimates over the design-based approach.
Model-based	No requirement (typically purposive sampling)	Model-dependent (based on the statistical model)	Introduced via the model of spatial variation	Superpopulation parameters	Model-bias, model-variance	aim is to the map the soil property of interest and want to use this map to predict values as precise as possible. efficiency over validity (when model is valid).

Once a sampling approach is chosen, the sampling design, including the required sample size and corresponding sampling units can be calculated. Only then can field sampling take place. Once sampled, a soil profile for Site B within the study truffière can be created, using data collected in the field. This soil profile can be used to compare areas of high truffle yield to specific soil qualities. If significant correlation occurs, then theories can be made about which soil properties potentially stimulate truffle yield at the study truffière. This research can assist in identifying options to optimise the productivity of the truffière, in order to maintain its benefits to the wider south-west region.

3.4.3 Chosen Sampling Strategy (Soil Sampling Plan)

This soil sampling plan was created under the guidance and assistance of Dr Ayalsew Zerihun and Professor Raphael Viscarra Rossel, Faculty of Science and Engineering, Curtin University, Western Australia. This sampling strategy takes a Design-Based approach, as the data created from the soil sampling is highly dependent upon the validity of the soil sampling results (Brus, 2020). Due to time and cost restraints associated with this research, and the sigificant restrictions of fieldwork inplace as a result of the COVID-19 pandemic, it was vital than any soil sampling strategy to be undertaken at the study truffiere had to be as efficient as possible. The Site B study area therefore needed to be separated into a number of soil sampling regions that best represented different areas of high, low and no truffle yield. These areas are best represented in the *Optimised Hot Spot Analysis* for the 2020 harvest season (Figure 3.8 from *3.3.3.3 -Variation in Truffle Yield - Site B*). This year of data was chosen as it best represents a "normal[2]" harvest year, as described by the manager of the study truffière.

To determine how the study area should be divided up into sampling regions, a new feature class, entitled *Truffle Density* was created. This feature class contained the location (Easting & Northing) of each tree within the Site B study area, as well as the corresponding number of truffles each tree produced over the harvest season (Appendix F). Before the *Truffle Density* feature class can be separated into soil sampling regions, the data was firstly reclassified to reduce the influence of 54 'outlier' trees over the clustering process. These trees produced significantly higher than average truffle yield and would therefore sway the clustering towards them. The outliers were identified by selecting all the trees that produced 50 or more truffles over the 2020 harvest season. Rather than deleting these trees altogether, they were reclassified so that the number field contained a maximum value of '50'. All other tree points were left the same.

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[2] "normal" in terms of soil, climate, growing and harvesting conditions, compared to the 2019 & 2021 harvest seasons.

An example of this reclassification for the first 5 data points in the *Truffle Density* feature class are shown below (Table 3.6).

Table 3.6 Reclassification of the ***truffle density*** feature class to reduce the influence of outlier points

Object ID	Shape	ID	Easting	Northing	Number	New Number
1	Point	1	413053.4366	6205550.916	160	50
2	Point	2	413055.8879	6205547.755	37	37
3	Point	3	413058.3391	6205544.594	32	32
4	Point	4	413060.7903	6205541.433	26	26
5	Point	5	413063.2416	6205538.272	60	50

This new feature class containing reclassified tree data was then clustered using the *Multivariate Clustering* tool within ArcGIS, using the 'Easting', 'Northing' and 'Number of Truffles Produced' as the analysis fields. These attributes were chosen as it allows the trees within the Site B study area to be clustered based on both their location and yield (i.e. the number of truffles they produced). The *Multivariate Clustering* tool separates the trees within the *Reclassified Truffle Density* feature class into *n* groups of equal variance, using the *k-means clustering* method. This method aims to maximise the within-group similarities and between-group differences, while minimising the within-cluster sum of squares (the distance between a tree data point and it's corresponding cluster, or region, centre) (Environmental Systems Research Institute (ESRI), 2021; Grekousis, 2020). The *k-means clustering* algorithm is stated as:

$$argmin \sum_{i=1}^{k} \sum_{x \in C_i} \| x - \mu_i \|^2$$

Where:

C_i is the set of observations that belong to cluster i;

μ_i is the mean of observations in C_i, and;

$k \leq n$ (the number of clusters is less than or equal to the number of data points) (Grekousis, 2020).

The *Multivariate Clustering* tool utilises unsupervised machine learning methods to determine natural clusters in the data (Environmental Systems Research Institute (ESRI), 2021). When run for the *Reclassified Truffle Density* feature class, the *Calinski-Harabasz pseudo-F-statistic* is calculated, in order to determine the optimal number of clusters for this study. This statistic measures the ratio of between-cluster variance to within-cluster variance and is determined by the following algorithm:

$$\frac{(\frac{R^2}{n_c - 1})}{(\frac{1 - R^2}{n - n_c})}$$

And:

$$R^2 = \frac{SST - SSE}{SST}$$

Where "SST is a reflection of between cluster differences and SSE reflects within-cluster similarity", and:

$SST = \sum_{i=1}^{n_c} \sum_{j=1}^{n_i} \sum_{k=1}^{n_v} (V_{ij}^k - \overline{V^k})^2;$

$SSE = \sum_{i=1}^{n_c} \sum_{j=1}^{n_i} \sum_{k=1}^{n_v} (V_{ij}^k - \overline{V_t^k})^2;$

n is the number of features;

n_i is the number of features in cluster i;

n_c is the number of clusters;

n_v is the number of variables used to cluster features;

V_{ij}^k is the value of the k^{th} variable of the j^{th} feature in the i^{th} cluster;

$\overline{V^k}$ is the mean value of the k^{th} variable, and;

$\overline{V_t^k}$ is the mean value of the k^{th} variable in cluster i (Environmental Systems Research Institute (ESRI), 2021)

The effectiveness of the *k-means clustering* process, using the number of clusters calculated by the *pseudo-F-statistic*, is determined through the coefficient of determination (R^2) value. This value reflects how much of the original variation in the data was retained after the *k-means clustering* process and is calculated from the following algorithm:

$$R^2 = \frac{(TSS - ESS)}{TSS}$$

Where:

TSS is the total sum of squares (calculated by squaring and summing deviations from the global mean value for an attribute) and;

ESS is the explained sum of squares (calculated the same as TSS, but every value is subtracted from the mean value for its corresponding cluster, then squared and summed) (Environmental Systems Research Institute (ESRI), 2021)).

The higher the R^2 value for a particular attribute (for example, 'Easting', 'Northing' or 'Number of Truffles Produced') the better that attribute is at discriminating features within the dataset. After running the *Multivariate Clustering* tool, the following summary statistics were returned (Figure 3.10).

Optimal number of clusters is 4 based on the highest pseudo F-statistic.

Variable-Wise Summary

Variable	Mean	Std. Dev.	Min	Max	R2
EASTING	412871.331620	139.466585	412590.223600	413178.449400	0.673006
NORTHING	6205334.637059	107.212194	6205043.121000	6205551.105000	0.621641
NUMBER	7.876615	8.745796	0.000000	50.000000	0.590843

Figure 3.10 Summary statistics for the k-means clustering of the *reclassified truffle density feature class*

Figure 3.10 indicates that the 'Easting', 'Northing' and 'Number' analysis fields do an acceptable job of separating the data, as indicated by the relatively high R^2 values. This *Multivariate Clustering* tool also returns a value of '4' as the optimal number of clusters for the data, based on the highest calculated *pseudo-F-statistic* (Figure 3.11). The result of the *k-means clustering* process on the *Reclassified Truffle Density* feature class (using 4 clusters), is shown below (Figure 3.12).

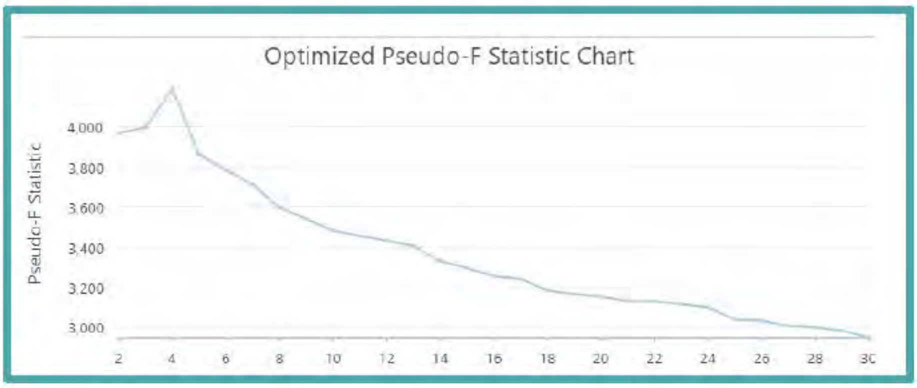


Figure 3.11 Optimised pseudo f-statistic chart for the clustering of the *reclassified truffle density feature class*

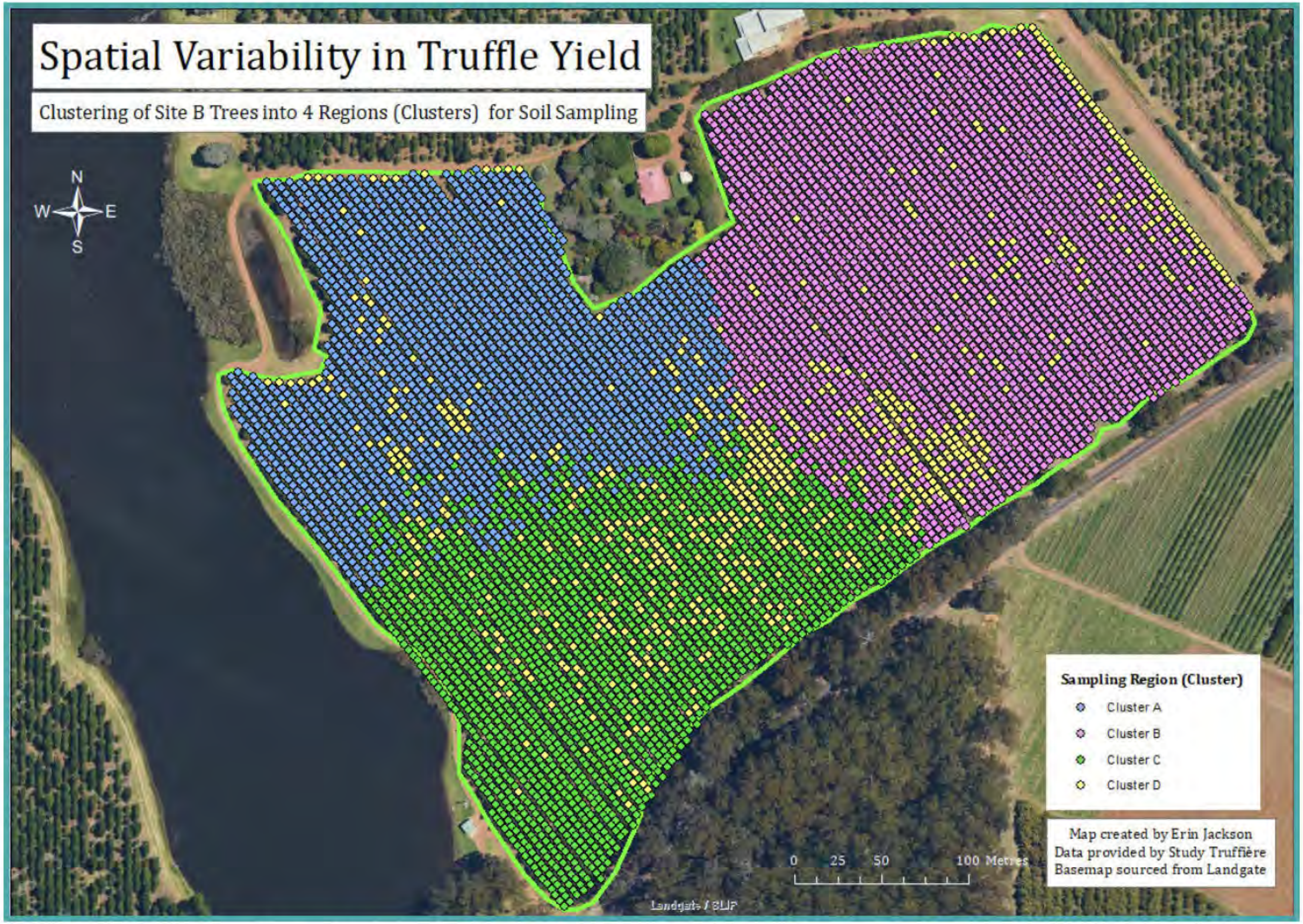


Figure 3.12 Harvested truffles (2020) separated into 4 cluster regions for soil sampling

The optimal number of soil sampling regions are also validated when compared to the *Optimised Hot Spot* for the 2020 harvest season (Figure 3.13). Clusters A, B & C are correlated with areas of little to no truffle yield, where Cluster D (yellow on the sampling regions map) is correlated with areas of high truffle yield (red on the optimised hot spot map). These clusters therefore represent a variety of conditions and are appropriate for this study.

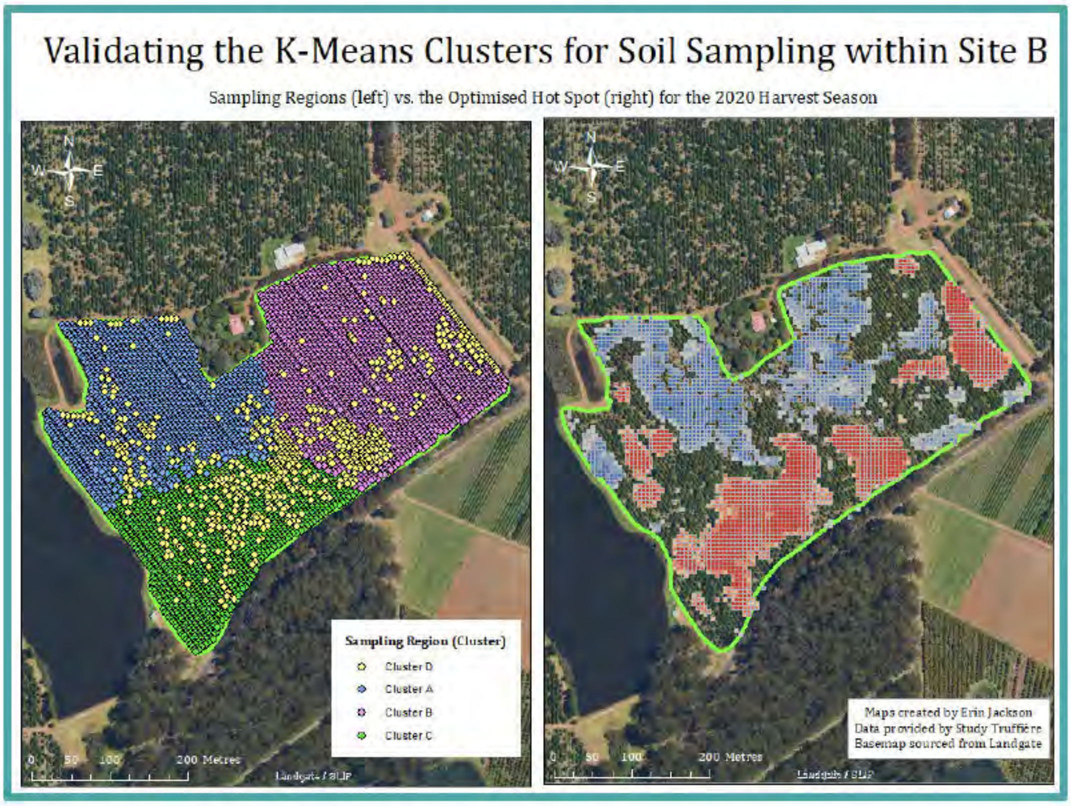


Figure 3.13 Validating the k-means clusters for soil sampling within Site B

Under the guidance of Dr Ayalsew Zerihun and Professor Raphael Viscarra Rossel from the Faculty of Science and Engineering at Curtin University, it was determined that approximately 40-50 soil samples taken from across the study area should be sufficient enough to create a soil profile that accurately represent the differing soil conditions across Site B. Due to time and cost restraints it was decided that 10 samples be taken per cluster region, with the sample trees from each cluster selected using a random sampling approach. Time constraints were also introduced as a result of the methodology involed in soil sampling (i.e. drilling in the soil). In order to avoid any damage to the crops, any fieldwork that was to be undertaken at the study truffiere could only take place in a limited period between late September and early October, directly after the seasons truffles had already been harvested in Site B.

In order to determine which trees within each cluster should be sampled to create the soil profile, the ArcGIS tool *Create Random Points* was utilised. This tool takes an extent region (e.g., Cluster A) and randomly selects a specified number of points within that region. This tool was run on each cluster region, selecting 10 random points (trees) from each cluster and ensuring a minimum allowed distance of 5m was selected (this decreases the chance of 2 sample trees being directly adjacent to each other). The selected points were then merged into a single feature class entitled *Soil Sampling Locations*. These points, in comparison to the *Optimised Hot Spot* for the 2020 harvest season, are shown below (Figure 3.14).

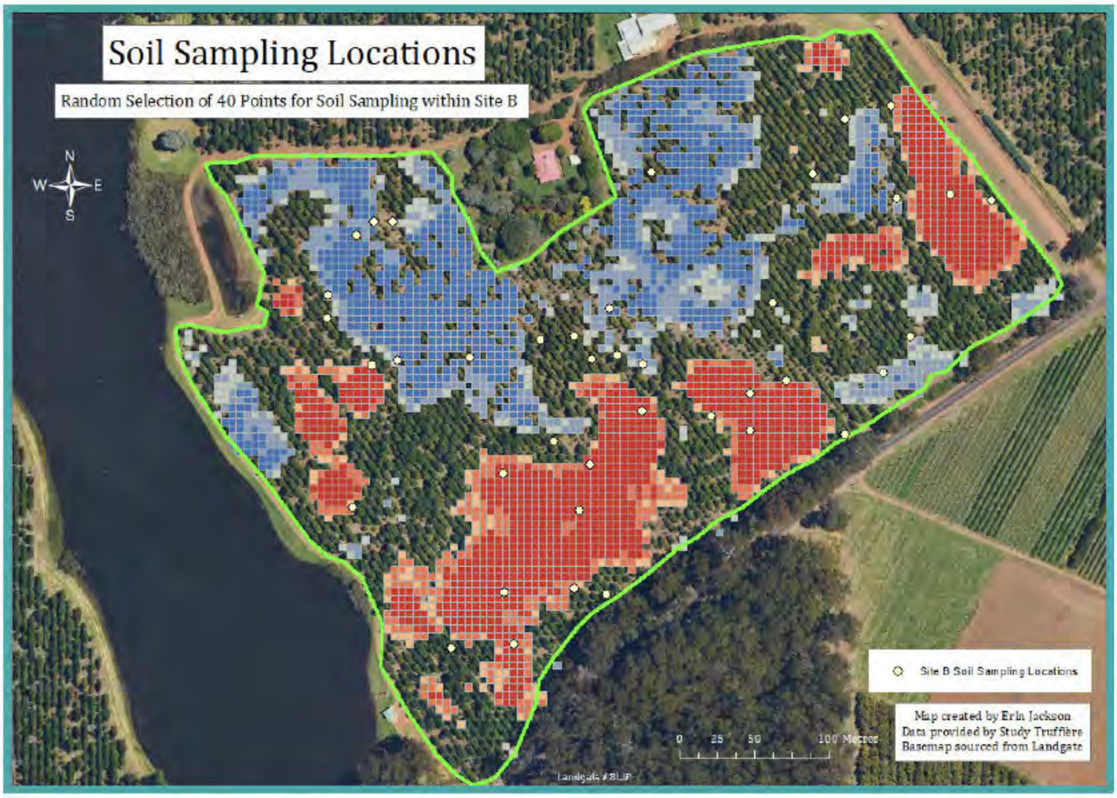


Figure 3.14 Random selection of 40 points (10 per cluster) for soil sampling within the Site B study area

Within the field, each of these sampling locations were found using a GPS and the corresponding sample location coordinates (Easting and Northing). 12-15 core samples were then taken approximately 1m from the base of the sample tree, at a depth of 0-10cm (Figure 3.15). These sample cores were then mixed until they the soil appeared to be well combined. A 200-500g sub-sample of this mixed soil was placed in a zip-lock bag and labelled with the corresponding sample number, tree ID number and location (Easting & Northing) (Appendix G) as well as the sampled date and time. These samples were then placed in a portable cooler box to keep the soil samples cool, as this reduces the chance of any microbial activity taking place and having a detrimental upon biological indicator measurements.

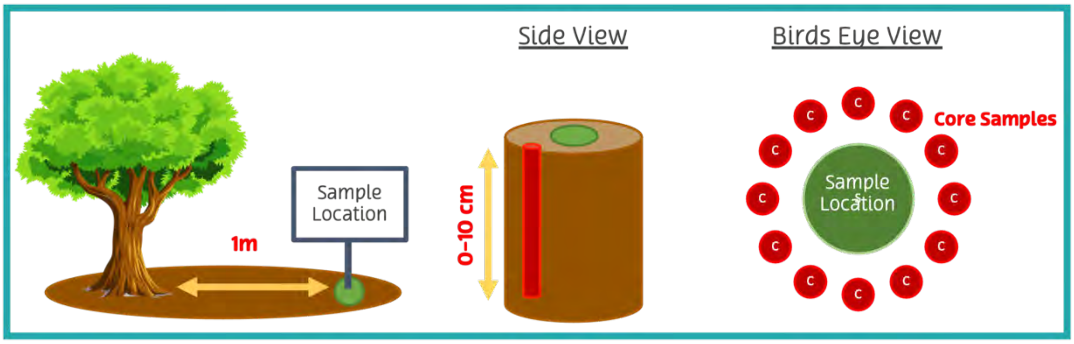


Figure 3.15 Soil sampling methodology

Immediately after fieldwork, these samples were taken to ChemCentre, at Curtin University, Western Australia, for further analysis. ChemCentre then analysed each sample and returned the sample pH, moisture content, particle size and nutrient ratios. This data was then used to create a soil profile for the Site B study area (this process is explored in greater detail in the next chapter (Chapter 4). This soil profile will be used to compare areas of high truffle yield to specific soil qualities. If significant correlation occurs, then theories can be extrapolated about which soil properties potentially stimulate truffle yield at the study truffière.

3. 5 Chapter Summary

Before this research was undertaken, little information regarding the study area, including site soil properties, was known. In order to uncover if particular soil properties (such as structure, pH and nutrient ratios), could stimulate truffle production, or whether spatial variability in truffle yield (i.e., the number of harvested truffles) was random and therefore due to individual tree performance, two key datasets were required; Firstly, the location of each harvested truffle and secondly, a soil profile for the Site B study area.

The location (latitude / longitude); the size (small / medium or large) and harvest time (day/month/year) was required to be collected and recorded against each, and every truffle picked in Site B over the harvest season. Appropriate methods used to obtain this locational data must have been able to record geographical location of each harvested truffle at a high speed, been accurate to less than 5m and available offline, due to the isolated location of the study area. GPS using a relative positioning technique failed to meet the accuracy required for this research, therefore locational data was collected using a mobile data acquisition technique, with the required data being provided directly by the study truffière.

In order to explore what factors (such as soil properties) could stimulate truffle production, firstly we determined whether a clear spatial variation in truffle yield existed in the Site B study area. The *Average Nearest Neighbour* and the *Optimised Hot Spot Analysis* both found that significant clustering and spatial variation existed within the study area for the 2019, 2020 and 2021 harvest seasons within Site B. It was therefore evident that certain trees within Site B produced more truffles than others and these trees changed from season to season. Due to issues with the utilisation of the mobile data capture device, there was severe underreporting of truffle data for the 2021 harvest season. The 2021 season's data will therefore not be used within the analysis section of this thesis as not to skew the analysis results.

The required soil profile was created through a soil sampling strategy developed using various ArcGIS analysis tools. Firstly, due to time and cost limitations, the study area was broken up into 4 sampling regions. These regions were determined through the *Multivariate Clustering* tool and the *k-means clustering* algorithm and were validated through the *Calinski-Harabasz pseudo-F-statistic*. 10 sampling trees were then randomly selected within each cluster using the *Create Random Points* tool. At each of these trees, 200-500 g of soil was collected from 12-15 core samples, taken at a depth of 0-10cm. These core samples were then provided to ChemCentre for further analysis. The returned data was then utilised to create a soil profile for the study area detailing the soil structure, nutrients, pH, and moisture content and drainage for Site B. This soil profile will be used to compare areas of high truffle yield to specific soil qualities. If significant correlation occurs, then theories can be extrapolated about which soil properties potentially stimulate truffle yield at the study truffière. These correlations are explored in the next chapter.

4 RESULTS & ANALYSIS

As discussed in Chapter 3, in order to uncover if soil structure, pH and nutrient ratios can stimulate truffle production, or whether spatial variability in truffle yield is random and therefore due to individual tree performance, two key datasets were required; Firstly, the location of each harvested truffle and secondly, a soil profile for the Site B study area. Within this research, the location, size and harvest time of each truffle collected over the 2019, 2020 and 2021 harvest seasons was obtained through the use of a mobile data acquisition technique. The *Average Nearest Neighbour* and the *Optimised Hot Spot Analysis* found that significant clustering and spatial variation existed within Site B for the 2019, 2020 and 2021 seasons. It is evident that certain trees within Site B are producing more truffles than others and these trees changed from season to season. As stated in Chapter 3 (Section *3.3.3.3 Variation in Truffle Yield Site B*), due to the underreporting of truffles harvested within Site B in the 2021 harvest season, this dataset will not be used in analysis as not to skew or influence the research results.

The previous chapter outlined how the soil samples were collected and provided to ChemCentre for further analysis. This chapter will explore the returned data and explain how it was used to create a soil profile for the study area. This profile will detail the soil structure, nutrients ratios, pH, and moisture content for the Site B study area and will be used to compare areas of high truffle yield to specific soil qualities. If significant correlation occurs between these two variables, then theories can be extrapolated about which soil properties potentially stimulate truffle yield at the study truffière.

4. 1 Results: Mapping Spatial Variability (Hot Spot Analysis)

4.1.1 Yield Data and Climatic Conditions for the Studied Harvest Seasons

In order to complete this research, the study truffière provided two csv files containing the truffle data for the 2019 and 2020 harvest seasons. Summary information for these datasets are shown below (Table 4.1).

Table 4.1 Summary statistics - study area truffle yield

Summary Statistics – Study Area Truffle Yield									
2019					2020				
Month	Small	Medium	Large	Total	Month	Small	Medium	Large	Total
May	441	194	8	643	*May*	-	-	-	-
June	10,125	2,875	186	13,186	*June*	19,810	6,018	360	26,188
July	12,832	2,025	138	14,995	*July*	23,181	7,632	583	31,396
August	3,590	429	16	4,035	*August*	1,438	547	11	1,996
Total	26,988	5,523	348	32,859	***Total***	44,429	14,197	954	59,580
Total (%)	82.13	16.81	1.06	100	*Total (%)*	74.57	23.83	1.60	100

The above figure shows that within Site B, each harvest season returns diverse yield results. As discussed in Chapter 2, truffle yield is highly dependent on specific climatic conditions. Warm summer temperatures are required to initiate truffle formation, while the mild, cooler winter temperatures trigger final maturation and develop the distinctive aromas of the fungus (Mathews & Mitchell, 2018). Additionally, the black truffle requires year-round rainfall, peaking in the winter months to facilitate high levels of production (Bradshaw, 2005). It can therefore be concluded that the 2019 and 2020 datasets indicate different climatic conditions over the 2 harvest seasons. These climatic differences were confirmed by both the Site B truffière manager and climate data sourced from the Bureau of Meteorology (Figures 4.1 and 4.2).

The 2019 harvest season provided a lower-than-average yield of only 32,859 (approx. 2,000kg) truffles over the harvest season (May to August). This low yield might be a result of the unusual climate conditions experienced that year. Figure 4.1 *Manjimup monthly mean rainfall (mm)* shows than in the lead up to the harvest season (January to May), the Site B study area didn't exceed the mean monthly rainfall of 50mm, far below the historical average for this time of year. Additionally, this low yield might be further explained by the higher-than-average temperatures experienced throughout the year. Figure 4.2 *Manjimup monthly mean maximum temperature (°C)* shows that the 2019 season experienced high-than-average mean maximum temperatures, especially in the winter months. According to the truffière manager, soils within Site B at this time did not drop below 12°C which is significantly higher than the below 10°C ideally required trigger final maturation. As smaller truffles within Site B would not have matured to their full capacity, these higher soil temperatures could explain the relatively low number of medium and large truffles produced over the 2019 harvest season.

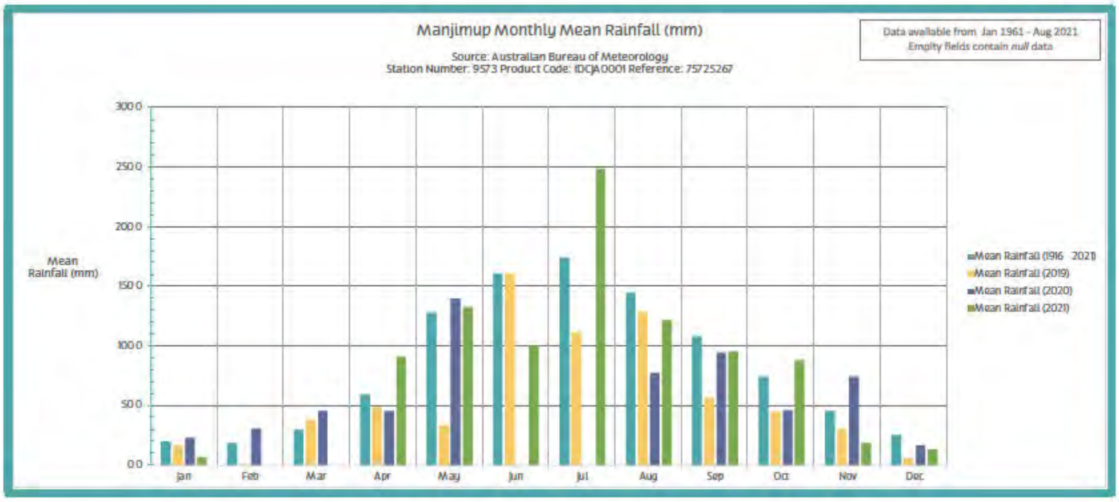


Figure 4.1 Manjimup monthly mean rainfall (mm)

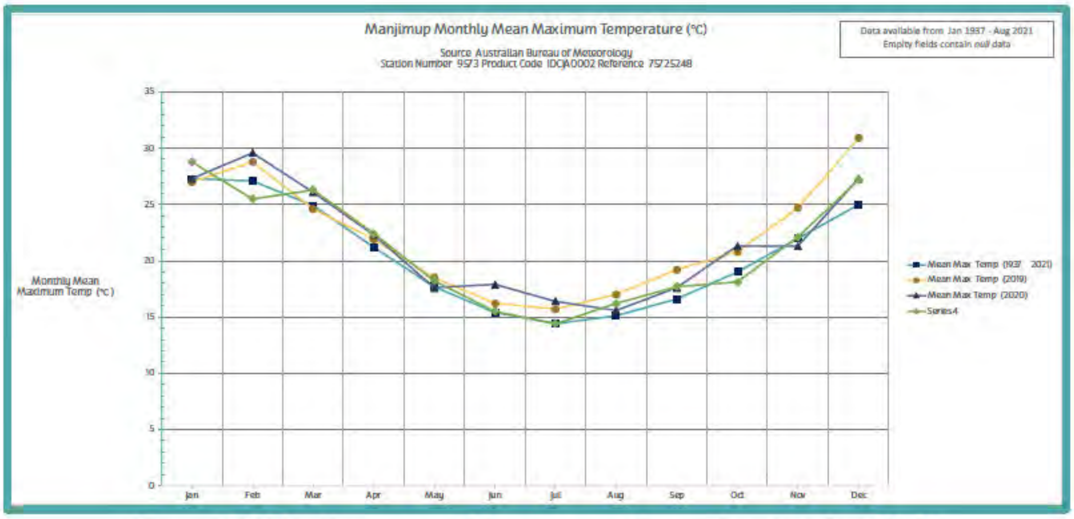


Figure 4.2 Manjimup monthly mean maximum temperature (°C)

Despite the 2020 harvest season not starting until later in the harvest year (June to August as opposed to the usual May to August), ideal climatic conditions resulted in a high yield of 59,580 (approx. 6000kg) of truffles for this season. According to the truffière manager, the 2020 harvest season data can be considered a "normal" harvest year, in which good climatic conditions resulted in an average number of truffles produced over a single season. Mean monthly rainfall was higher and more consistent than the previous year, especially during the summer months (Jan March) (Figure 4.1) allowing more truffles to form and grow than the previous 2019 season. This rainfall might also explain the higher percentage of medium and large truffles produced in 2020 compared to the 2019 season. Additionally, this typical yield result experienced in the 2020 harvest season may be explained by the warmer than average summers and relatively cool winter temperatures which would have facilitated ideal growing conditions (Figure 4.2).

Due to the underreporting of Site B truffles for the 2021 harvest season, the corresponding dataset did not accurately reflect the yield results for 2021 and could therefore not be used in this analysis. According to the truffière manager, the 2021 harvest season produced the largest yield of the three years studied, with a higher-than-average number of harvested truffles falling into the "medium" and "large" size categories, with approximately 10,000kg of truffles being harvested from Site B between May and August 2021. This high yield might be a result of the high rainfall experienced in November 2020 during the initial formation of the truffles to be harvest in 2021. Additionally, the 2021 harvest season experienced the coolest winter temperatures (June and July) of the 3 seasons studied in this research (Figure 4.2). These cool winter temperatures would have triggered final maturation, further allowing a higher-than-average yield of truffles to form over the 2021 harvest season.

4.1.2 Optimised Hot Spot Analysis Results for the Studied Harvest Seasons

As discussed in Chapter 3 (Section *3.3.2 Average Nearest Neighbour Ratio*) the 2019 and 2020 harvest seasons ANN ratios indicate that there is evidence of spatial variability in the harvested truffles within the Site B study area. The corresponding ANN reports (Appendix E) show a significant level of clustering within these two datasets, with a high confidence level that this clustering is not due to a random process. Chapter 3 (Section *3.3.3.3 Variation in Truffle Yield Site B*), and the *Optimised Hot Spots* for the 2019 and 2020 harvest seasons (Figures 3.7 and 3.8 in the previous chapter) demonstrate that spatial variability in truffle yield does exist within the Site B study area. Certain trees within the Site B truffière (known as "hot" trees) produce more truffles over a harvest season than others, (as evident in Table 4.1 *Summary statistics - study area truffle yield* above) and these areas change from season to season.

Figure 4.3 illustrates the changes in hot spots, and therefore the shift in spatial variability between the 2019 and 2020 harvest seasons. Within the 2019 harvest season, three main hot spots can be identified; hot spot A in the lower SW corner of the site, hot spot B that along the of the southern border and hot spot C in the SE corner that runs along the eastern border of the site (Figure 4.3). Between 2019 and 2020 these hot spots stayed relatively consistent, with the addition of hot spot D in the upper western border of Site B (Figure 4.3). Between these two years, hot spot A grew significantly, spreading further towards the NW corner, and retreating from the SW corner of the site. Hot spot B moved further towards the SW corner and moved further to the centre of Site B, while hot spot C shifted further north. Further comparisons between these years can be made when analysing the change in cold spots for these harvest years (Figure 4.4).

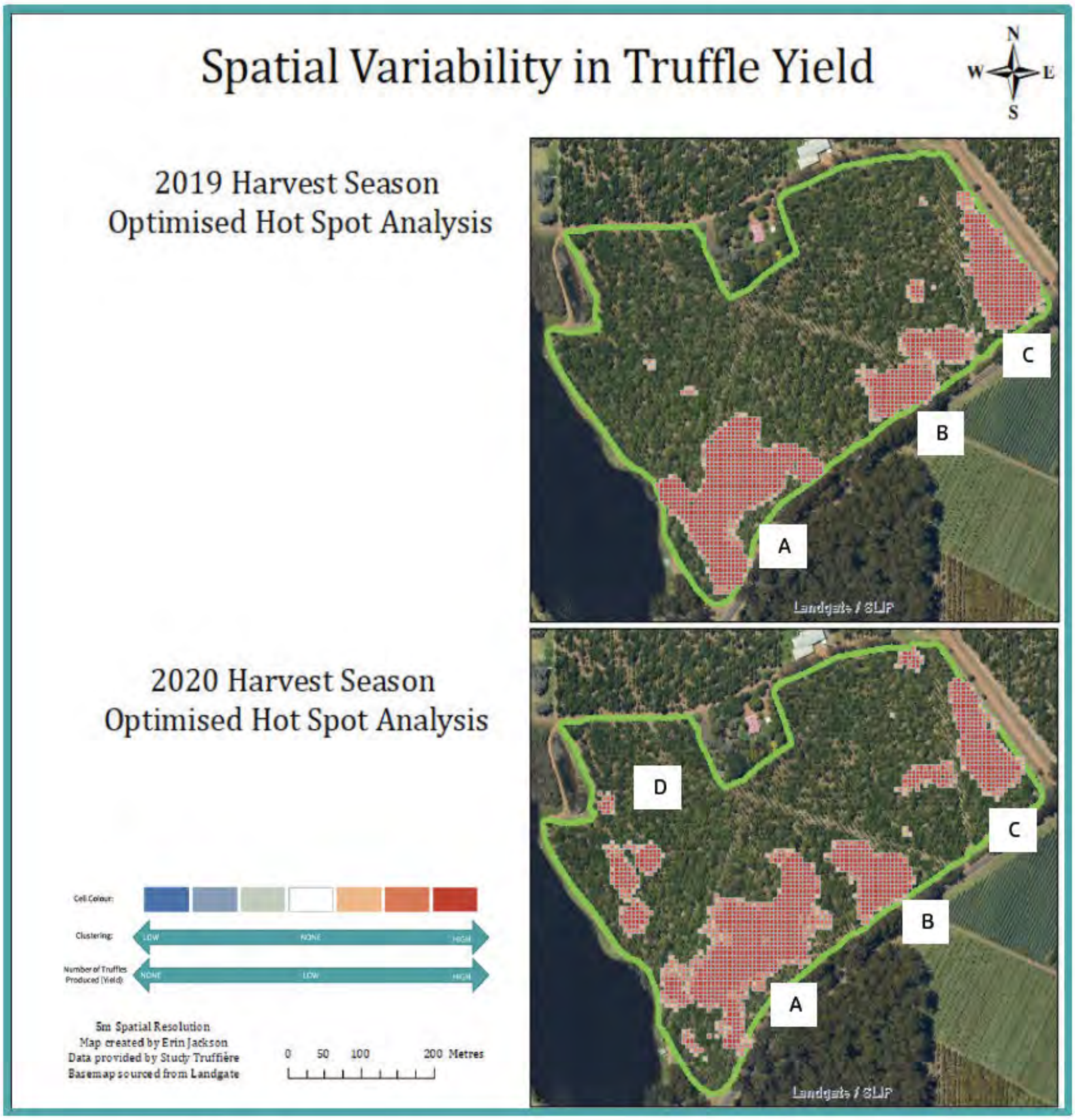


Figure 4.3 Site B Optimised Hot Spots for the 2019 and 2020 harvest seasons

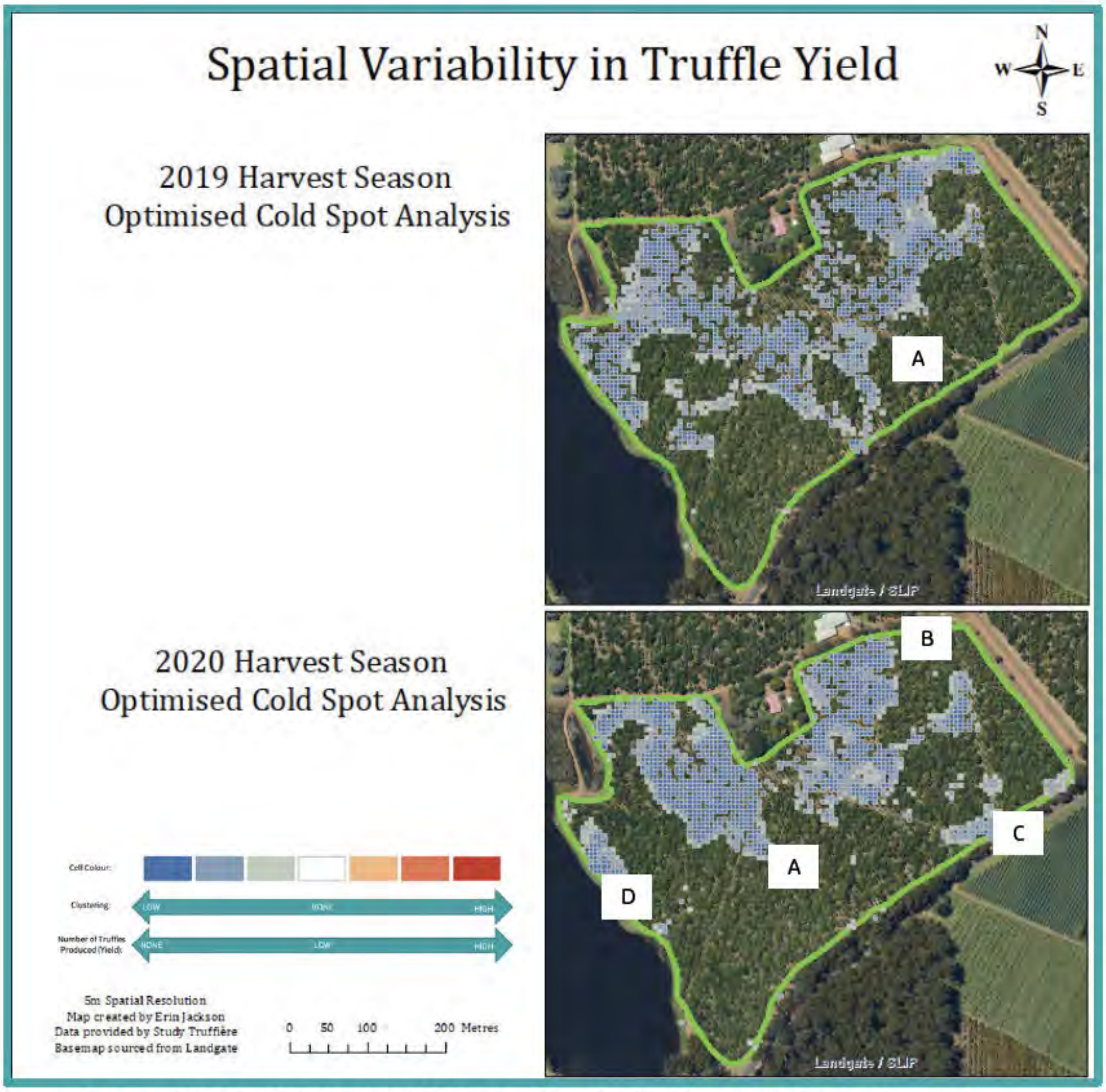


Figure 4.4 Site B Optimised Hot Spots for the 2019 and 2020 harvest seasons

Figure 4.4 (above) illustrates the changes in cold spots between the 2019 and 2020 harvest seasons. In comparison to Figure 4.3 (the changes in hot spots between the 2019 and 2020 harvest seasons) it is evident that certain hot spots in 2019 have shifted to cold spots in 2020. For example, the lower right section of hot spot B and the bottom section of hot spot C from the 2019 *Optimised Hot Spots* have turned blue in the 2020 *Optimised Cold Spots.* These changes are illustrated below (Figure 4.5).

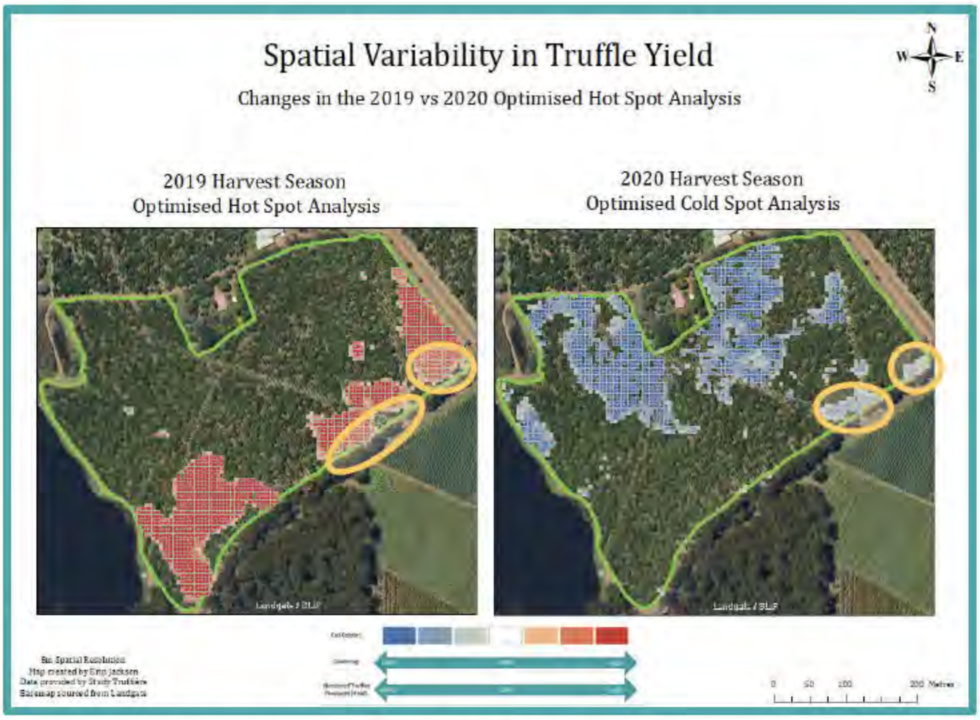


Figure 4.5 2019 Optimised Hot Spot Analysis vs. 2020 Optimised Cold Spot Analysis

Additional conclusions regarding the change in spatial variability of truffles within Site B can be made from Figure 4.4 *Site B Optimised Hot Spots for the 2019 and 2020 harvest seasons*. Firstly, the cold spots within Site B remained fairly consistent between the two studied harvest seasons, and these cold spots tend to be located in the northern sector of the site (Figure 4.4). Within the 2019 harvest season there is one continuous cold spot (cold spot A) that spread from the both the NW and NE corners of the study area (Figure 4.4). The two segments of this cold spot meet in the centre of Site B, before spreading southwards to touch the southern border of the study area (Figure 4.4). The 2020 harvest season saw this cold spot split in two; cold spot A to the NW and cold spot B along the northern edge of the study area. Additionally, the 2020 harvest season saw the addition of a cold spot in the SE corner (cold spot C) and on the western border of Site B (cold spot D) (Figure 4.4).

The hot and cold spots created in the *Optimised Hot Spot Analysis* for the 2019, and 2020 harvest seasons found that significant clustering and spatial variation existed within Site B. It is therefore evident that certain trees within Site B are producing more truffles than others and these trees change from season to season. As outlined in Chapter 2, it is expected that this spatial variability is highly dependent upon the quality of the soil that that the truffles grow in. If significant correlation exists between these hot and cold spots, and the soil conditions within Site B, then theories can be extrapolated about which soil properties potentially stimulate truffle yield at the study truffière. In order for these comparisons to be made, a soil profile containing detail information regarding the soil structure, nutrients, pH, and moisture content and drainage for the Site B study area was required. The creation of this profile is outlined in the next section.

4. 2 Results: Soil Profile (Soil Sampling Survey)

Fieldwork undertaken at the study area in September 2021 collected 40 soil samples that accurately represent the differing soil conditions across Site B. The location of these samples, and their corresponding sample number is available in Appendix H. These samples were taken to ChemCentre, at Curtin University, Western Australia, who analysed each sample and returned the samples soil properties including the moisture content, soil pH, particle size (soil structure) and nutrient ratios. The completed soil analysis provided by ChemCentre is available in Appendix I. The "box and whisker plots" for each soil property is available in Appendix J. This section will outline the summary statistics and provide the soil profile for each of these soil qualities.

As outlined in Chapter 2, it is expected that this spatial variability is highly dependent upon the quality of the soil that the truffles grow in. In order for conclusions to be made about which soil properties potentially stimulate truffle yield at Site B, a soil profile containing detail information regarding the soil structure, nutrients, pH, and moisture content and drainage for the Site B study area was required. These soil profiles were created by classing the soil data into 4 groups using the *Natural Breaks (Jenks)* method. This number of classes (4) was chosen as it allows for easier comprehension of the profile, without generalising the data. The *Natural Breaks (Jenks)* method divides the dataset into the 4 classes based on natural groupings (patterns) inherent in the data.

4.2.1 Moisture Content

Within the soil analysis, ChemCentre determined the moisture content of the soil as a percentage of its oven-dried weight. This method is based on "*removing soil moisture by oven-drying a soil sample until the weight remains constant; the moisture content (%) is calculated from the sample weight before and after drying*" (Craze, 1990). For the soil within Site B, a sub-sample of each soil sample collected from Site B was weighed at 40°C. This sub-sample was then heated to 105°C, until the weight remained constant. The sub-sample was then cooled and reweighed to give the percentage of moisture content. The value recorded at 105°C returns the soils highest moisture content.

Table 4.2 Site B soil moisture (%) – summary statistics

Site B Soil Moisture (%) – Summary Statistics			
Moisture Content @ 105°C		Moisture Content @ 40°C	
Statistic	**Value (%ar)**	**Statistic**	**Value (%ar)**
Count	40	Count	40
Minimum	20	Minimum	16.6
Maximum	32.6	Maximum	30.4
Mean (Average)	27.13	Mean (Average)	24.56
Mode (Most Frequent Value)	25.9, 30.2, 31.1, 27.2 & 29.9	Mode (Most Frequent Value)	27, 23.7 & 18.2
Standard Deviation	3.4	Standard Deviation	3.44

Table 4.2 outlines the summary statistics for the moisture content of the soil within the Site B study area. The 40 soil samples taken from Site B returned a minimum moisture value of 20% (105°C) and 16.6% (40°C), with both values recorded at sample location 20. Sample 33 returned the highest moisture content at both 105°C (32.6%) and 40°C (30.4%). The average moisture content for Site B at 105°C was recorded as 27.13%, and 24.56% for moisture content at 40°C, both with a standard deviation of 3.4. The moisture soil profile (i.e. how the moisture content changes over the Site B study area) at 105°C is available in Figure 4.6 below. More details regarding soil moisture and the corresponding truffle yield over the Site B study area is available in *4.3 Linking high truffle yield to soil properties.*

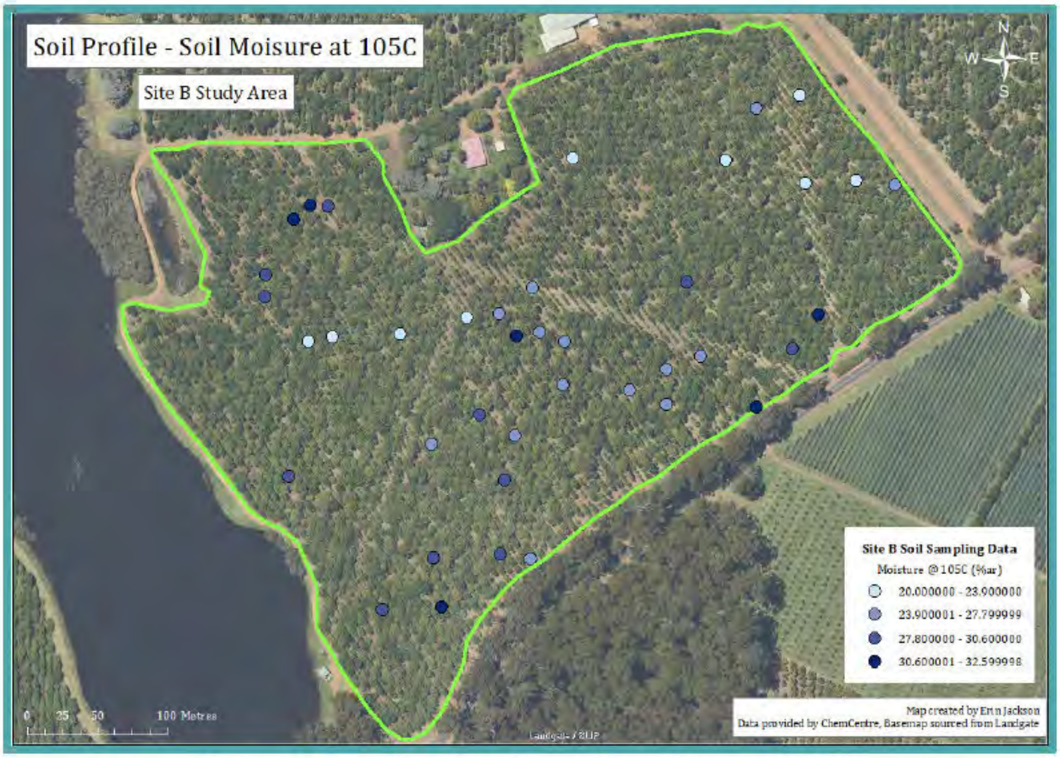


Figure 4.6 Site B soil profile - soil moisture at 105°C

4.2.2 pH Levels

ChemCentre determined the pH for each sample by firstly air drying all 40 soil samples taken within the study area. A sub-sample of these samples was then taken and mixed with five times its weight of a dilute concentration (0.01M) of calcium chloride ($CaCl_2$) (Lake & New South Wales Agriculture, 2000). The sample was then shaken for 1 hour and the pH was measured using an electrode (Lake & New South Wales Agriculture, 2000). Table 4.3 shows the summary statistics for the pH values measured across the Site B study area.

Table 4.3 Site B soil pH - summary statistics

Site B Soil pH – Summary Statistics	
Statistic	**Value**
Count	40
Minimum	5.2
Maximum	7.5
Mean (Average)	6.98
Mode (Most Frequent Value)	7.2
Standard Deviation	0.467

The 40 soil samples taken from Site B returned a minimum pH value of 5.2 (sample location 3) and a maximum pH value of 7.5 (sample locations 13 & 28). 17.5% of the samples recorded a pH value of 7.2 (sample locations 11, 12, 15, 34, 21, 24 & 16). The average recorded pH for Site B was 6.98, with a standard deviation of 0.467. The soil profile for pH is available in Figure 4.7 below. More detail regarding soil pH and the corresponding truffle yield over the Site B study area is available in *4.3 Linking high truffle yield to soil properties*.

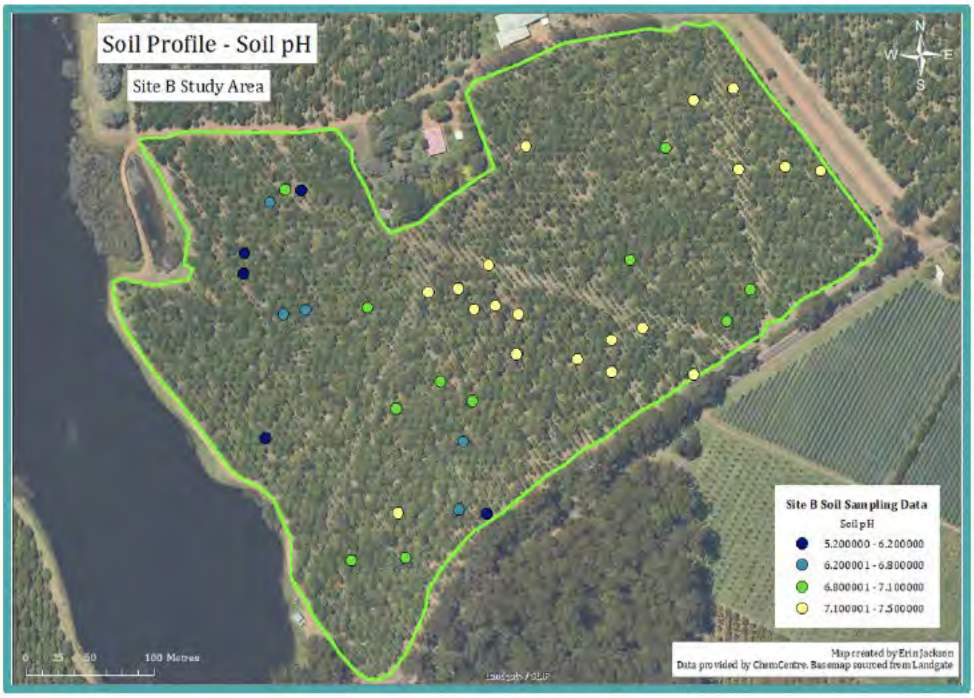


Figure 4.7 Site B soil profile - soil pH

4.2.3 Soil Structure

Chem Centre measured the structure of the soil within Site B, using the methods outlined in the Australian Standard AS1289.C6.3: Determination of the particle size distribution of a soil stand method of fine analysis using a hydrometer. This method involves passing the pre-treated soil samples through various grained sieves to separate the soils into various particle sizes (DSNR., 2002). Particles less than 0.002mm fall under the clay structure, particles between 0.002 and 0.02mm fall under the silt structure, and particles between 0.02 and 2.0mm are classified as sand. For a more detailed methodology used in determining soil structure, please see the NSW Department of Sustainable Natural Resources 2002 report, *Soil Survey Standard Test Method: Particle Size Analysis* (DSNR., 2002).

Table 4.4 Site B soil structure - summary statistics

Site B Soil Structure – Summary Statistics					
Sand (%)		Silt (%)		Clay (%)	
Statistic	**Value (%)**	**Statistic**	**Value (%)**	**Statistic**	**Value (%)**
Count	40	Count	40	Count	40
Minimum	60	Minimum	12	Minimum	5
Maximum	80.5	Maximum	23	Maximum	19
Mean (Average)	74.19	Mean (Average)	16.96	Mean (Average)	8.85
Mode (Most Frequent Value)	76	Mode (Most Frequent Value)	17	Mode (Most Frequent Value)	7
Standard Deviation	4.22	Standard Deviation	2.54	Standard Deviation	2.95

Table 4.4 outlines the summary statistics for the soil structure ratios measured across the Site B study area. The 40 soil samples taken from Site B returned a minimum sand content value of 60% (sample location 21) with a maximum sand content value of 80.5% (sample location 3). Seven of the samples recorded a sand content value of 76% (sample locations 11, 1, 4, 20, 9, 40 & 25). The average recorded sand content for Site B was 74.19%, with a standard deviation of 4.22. The minimum silt content value from the 40 samples was 12% (sample location 36) with the maximum silt content at 23% (sample location 30). 17.5% of the samples measured a silt content of 17% (sample locations 9, 11, 4, 23, 26, 38 & 25). The average silt content across Site B was measured at 16.96% with a standard deviation of 2.54.

All 40 samples returned a similar structure ratio, in which they contained a higher percentage of sand to silt and a low percentage of clay content. The minimum clay content (5%) was recorded at sample location 12, with the highest clay content (19%) recorded at sample location 21 (which also sampled the lowest sand content across Site B). The structure soil profile is available below (Figure 4.8). More detail regarding the soil structure and the corresponding truffle yield over the Site B study area is available in *4.3 Linking high truffle yield to soil properties.*

Figure 4.8 Site B soil profile - soil structure

4.2.4 Nutrient Ratios

Within this soil analysis, ChemCentre determined the amount of plant-available nutrients (including sodium, nickel, cobalt, copper, iron, potassium, magnesium, manganese, molybdenum, aluminium, arsenic, boron, calcium, cadmium, sulphur, selenium, zinc, phosphorus and lead) within the Site B study area, using the Mehlich No. 3. Soil test extractant method (Mehlich, 1984). This method uses an extracting solution to obtain content (by weight) of each nutrient within the soil sample. Concentration is reported as mass of the chemical in micrograms (mg) per mass of soil (kg) (i.e. mg/kg). For more details about this method please see Mehlich (1984). Total Nitrogen was measured by combustion using the inhouse method S57, returning a % of total nitrogen present within the soil sample.

This study will only focus on those nutrients that play a role in the production of the black truffle including phosphorous, calcium, magnesium, nitrogen, potassium, sulphur, boron, copper, iron, manganese, zinc and sodium. It should be noted that within this analysis results that are reported as ">" are outside the linear range of the calibration and outside the scope of the collection method and these results should be used as a guide only. As calcium is one of the nutrients that falls into this category, it will not be used to determine the influence of soil nutrients over truffle yield within the study area. The soil profile for each nutrient and the summary statistics for these nutrients are show below (Figures 4.9 - 4.10 & Table 4.5).

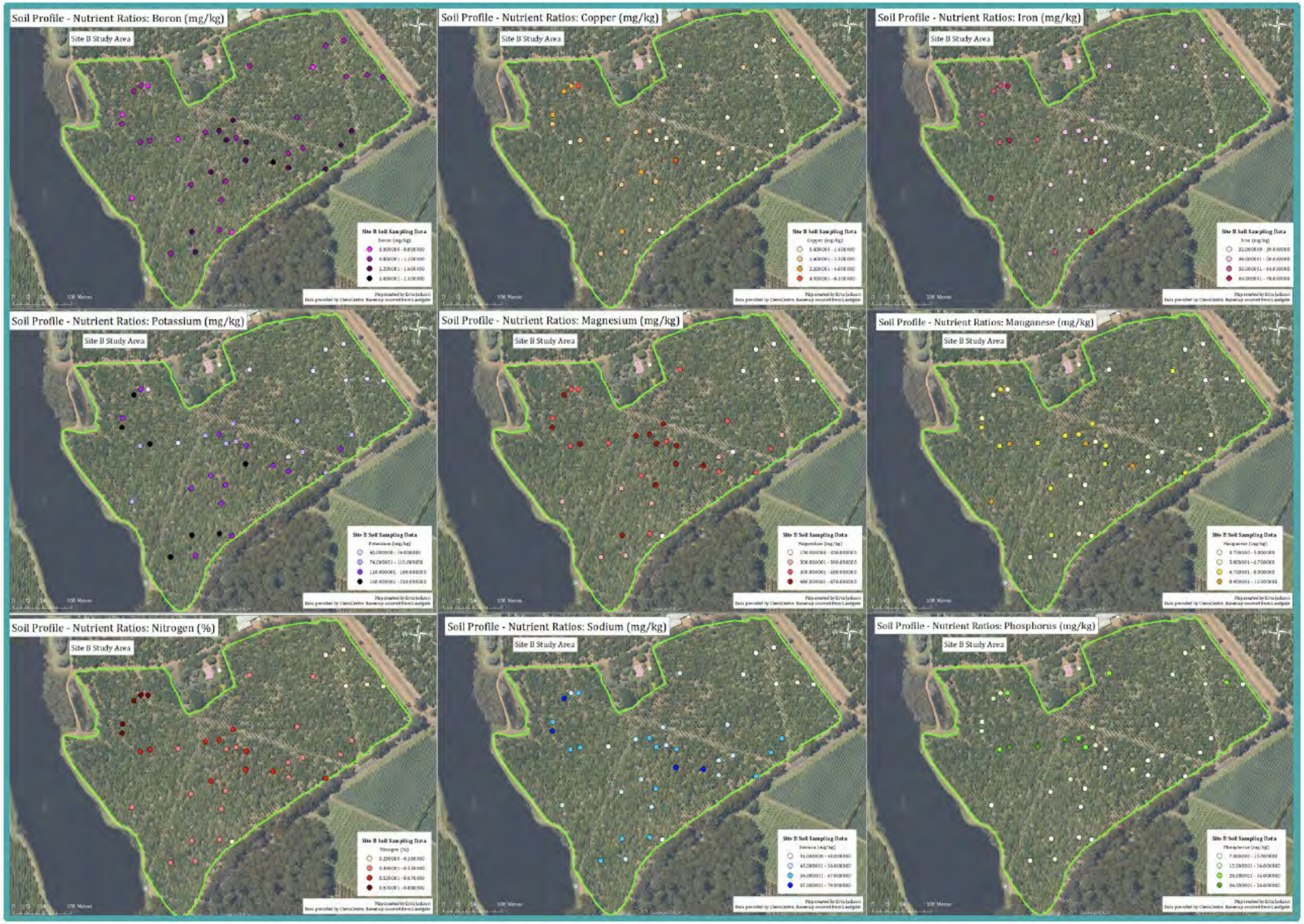


Figure 4.9 Site B soil profile - soil nutrients (part one). Caption reads: *Map created by Erin Jackson. Data provided by ChemCentre, basemap from Landgate.*

Figure 4.10 Site B soil profile - soil nutrients (part two). Caption reads: *Map created by Erin Jackson. Data provided by ChemCentre, basemap from Landgate.*

Table 4.5 Site B soil nutrients - summary statistics

Site B Soil Nutrients – Summary Statistics							
Boron (B)		**Calcium (Ca)**		**Copper (Cu)**		**Iron (Fe)**	
Statistic	Value (mg/kg)	Statistic	Value (mg/kg)	Statistic	Value (mg/kg)	Statistic	Value (mg/kg)
Count	40	Count	40	Count	40	Count	40
Minimum	0.5	Minimum	1000	Minimum	0.4	Minimum	31
Maximum	2.3	Maximum	>5500[3]	Maximum	6.3	Maximum	78
Mean (Average)	1.15	Mean (Average)	5197.5	Mean (Average)	1.83	Mean (Average)	46.8
Mode (Most Frequent)	1.1	Mode (Most Frequent)	>5500	Mode (Most Frequent)	1.8	Mode (Most Frequent)	43
Standard Deviation	0.32	Standard Deviation	869.27	Standard Deviation	1.25	Standard Deviation	12.1
Potassium (K)		**Magnesium (Mg)**		**Manganese (Mn)**		**Nitrogen (N)**	
Statistic	Value (mg/kg)	Statistic	Value (mg/kg)	Statistic	Value (mg/kg)	Statistic	Value (%)
Count	40	Count	40	Count	40	Count	40
Minimum	40	Minimum	150	Minimum	3.7	Minimum	0.25
Maximum	210	Maximum	670	Maximum	13	Maximum	0.88
Mean (Average)	117.98	Mean (Average)	417	Mean (Average)	6.59	Mean (Average)	0.51
Mode (Most Frequent)	130	Mode (Most Frequent)	520	Mode (Most Frequent)	5.8	Mode (Most Frequent)	0.45
Standard Deviation	47.7	Standard Deviation	106.5	Standard Deviation	1.93	Standard Deviation	0.15

[3] Results that are reported as ">" are outside the linear range of the calibration and outside the scope of the method. Results should only be used as a guide and consideration should be given to a more specific test method if the actual "value" need to be determined (Bolland, Allen & Walton, 2002) as stated in ChemCentre Report of Examination.

Sodium (Na)		Phosphorous (P)		Sulphur (S)		Zinc (Zn)	
Statistic	Value (mg/kg	Statistic	Value (mg/kg)	Statistic	Value (mg/kg)	Statistic	Value (mg/kg)
Count	40	Count	40	Count	40	Count	40
Minimum	31	Minimum	7	Minimum	9	Minimum	0.4
Maximum	79	Maximum	56	Maximum	20	Maximum	3.6
Mean (Average)	54.7	Mean (Average)	20.0	Mean (Average)	14.25	Mean (Average)	1.43
Mode (Most Frequent)	52	Mode (Most Frequent)	12	Mode (Most Frequent)	14	Mode (Most Frequent)	2.1
Standard Deviation	11.9	Standard Deviation	11.3	Standard Deviation	2.92	Standard Deviation	0.64

The 40 soil samples taken from Site B returned an average boron content of 1.15mg/kg, with the highest content (2.3 mg/kg) recorded at sample location 13, and the lowest content (0.5mg/kg) recorded at sample location 3. The highest and lowest content of magnesium where also recorded at these locations respectively, with the highest content of sulphur (20mg/kg) also recorded at sample location 13 (equal with sample location 24) and the lowest content of zinc (0.4mg/kg) also recorded at location 3. Magnesium provided the highest standard deviation (869.27) out of all of the nutrients measured (not including calcium, due to the inaccuracy of the data). Sample location 7 recorded the highest content of iron (78mg/kg) and manganese (13mg/kg), but the lowest content of copper (0.4mg/kg) (equal with sample location 21).

Sample location 37 in the top NW of the study area returned the lowest content values for both sodium (31mg/kg) and sulphur (9mg/kg). This section of the study area also produced the highest concentrations of copper (6.3mg/kg recorded at location 35) and zinc (3.6 mg/kg recorded at location 39), and the lowest concentration of nitrogen (0.25% at location 46). The highest content of potassium (210mg/kg) were recorded at adjacent locations (sample locations 4 and 5), while the lowest content (40mg/kg) was recorded at locations 40 and 35. More detail regarding each nutrient and the corresponding truffle yield over the Site B study area is available in *4.3 Linking high truffle yield to soil properties*.

4. 3 Linking High Truffle Yield to Soil Properties

As discussed in Chapter 2, in order to produce the *Tuber melanosporum*, specific soil qualities are required. Ideally, for a high production of the black truffle, the soils must have:

(a) A pH level between 7.2 and 8.3 (7.9pH is ideal);
(b) A loose structure (soils with high gravel content, e.g. sandy clay loam are ideal); and,
(c) A specific nutrient ratio e.g., a "*relatively balanced ratio of… phosphorus, calcium, magnesium, nitrogen, potassium, sulphur with trace elements of boron, copper, iron, manganese and zinc*", high concentrations of plant available calcium and magnesium, moderate levels of phosphorus and little concentration of sodium (Hall et al., 2007).

This section will explore how each of the soil attributes measured by ChemCentre (e.g., moisture content, pH levels, structure and nutrient ratios) relate to the truffle yield measured over the studied harvest seasons at the Site B study area, using soil profiles created in the previous section and the 2019 and 2020 *Tree Density Optimised Hot Spot Analysis* (Figures 4.9 and 4.10). These figures cluster each tree within the Site B, using the number of truffles the tree produced in the harvest year as the analysis field, into statistically significant hot and cold spatial clusters. These clusters are mapped using the Getis-Ord Gi* statistic outlined in Chapter 3 (Section *3.3.3 Optimised Hot Spot Analysis)*. The hot and cold spots identified in Figures 4.9 and 4.10 closely resemble the hot and cold spots identified in the 2019 and 2020 *Optimised Hot Spot Analysis* (Figures 4.3 and 4.4 above).

Table 4.3 *Site B soil sampling locations and their corresponding 2019 and 2020 Tree Density Optimised Hot Spot Analysis values* will be used to assist in linking each soil sample to areas of high, low or little truffle yield. If significant correlation exists between these hot and cold spots, and the soil conditions within Site B, then theories can be extrapolated about which soil properties potentially stimulate truffle yield at the study truffière.

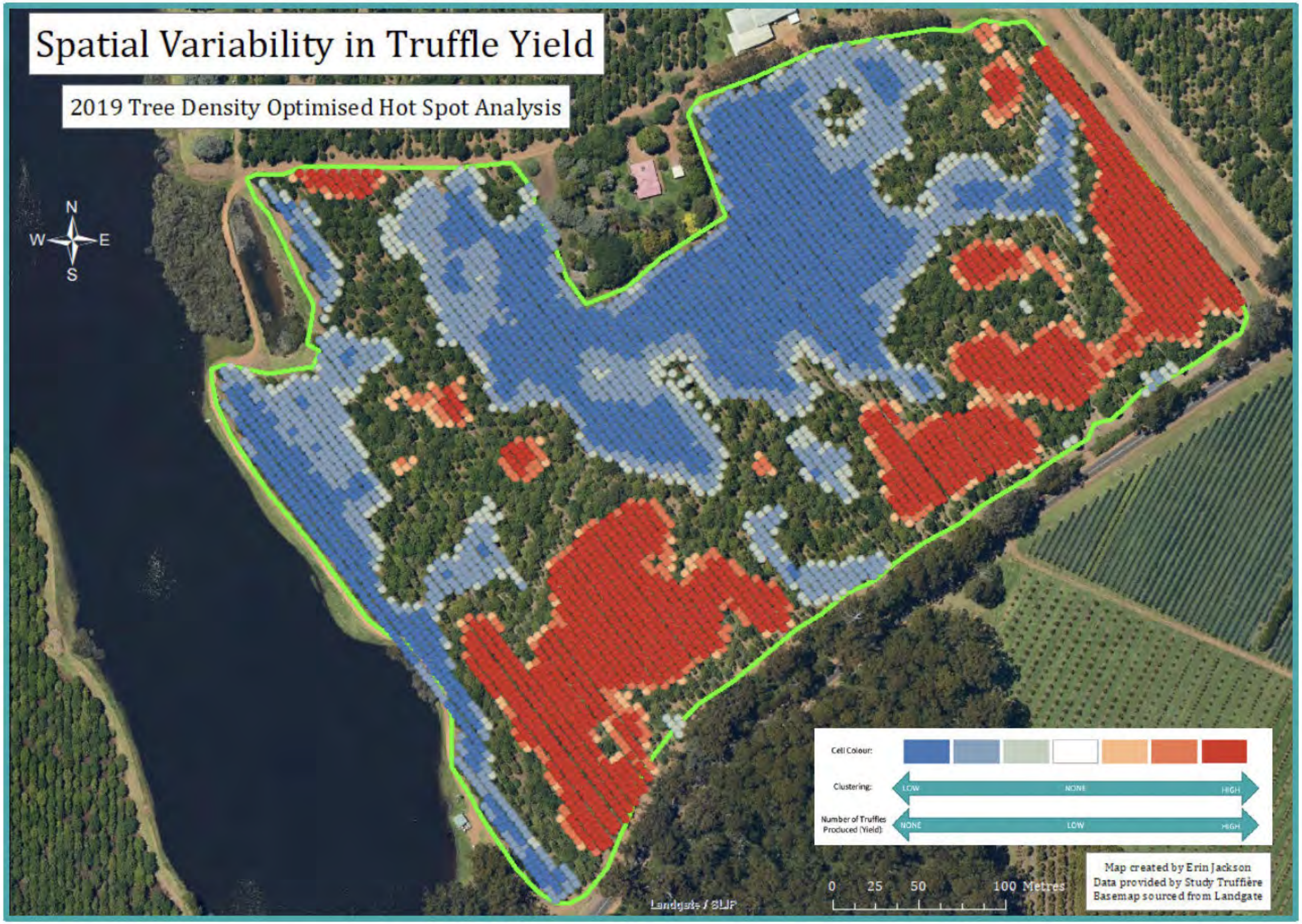


Figure 4.11 Tree Density Optimised Hot Spot for the 2019 harvest season

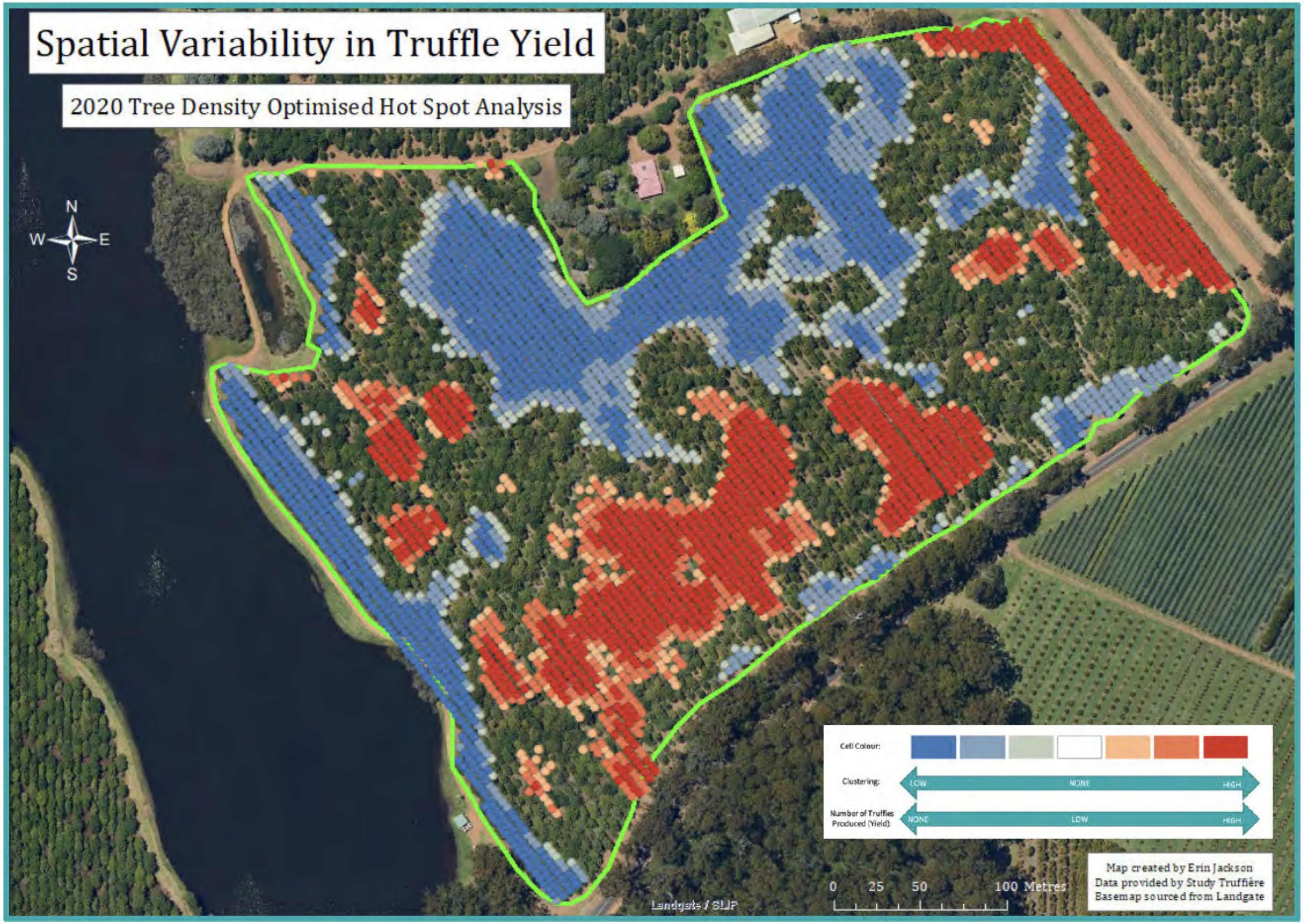


Figure 4.12 Tree Density Optimised Hot Spot for the 2020 harvest season

Table 4.6 Site B soil sampling locations and their corresponding 2019, 2020 and 2021 *Optimised Hot Spot Analysis* values

Sample Location	Tree ID	Easting	Northing	2019 Density OHS	Tree Yield (2019)	2020 Density OHS	Tree Yield (2020)
1	6880	412769.115	6205133.61	3	5	3	15
2	6316	412811.456	6205135.9	0	11	0	11
3	5081	412874.518	6205169.87	0	1	0	0
4	6074	412805.155	6205171.02	3	5	3	9
5	5364	412852.807	6205173.52	3	4	0	9
6	4697	412856.217	6205226.32	2	3	0	3
7	6765	412702.396	6205228.94	0	6	2	5
8	5149	412804.136	6205251.96	3	7	3	8
9	4250	412863.227	6205257.68	0	0	3	22
10	4418	412838.558	6205273.48	2	3	2	13
11	2370	413035.91	6205278.55	0	0	-1	0
12	2936	412972.042	6205280.7	1	2	1	12
13	3093	412945.566	6205290.47	-1	0	0	20
14	3469	412898.173	6205294.26	2	3	3	25
15	2769	412971.662	6205305.71	3	7	3	16
16	2489	412996.301	6205314.6	3	6	3	16
17	1741	413061.653	6205319.81	0	9	-1	2
18	5611	412715.843	6205325.29	2	16	3	48
19	3244	412899.179	6205325.72	0	4	2	7
20	5315	412732.985	6205328.44	0	4	2	13
21	3512	412864.759	6205329.24	-1	0	2	20
22	4575	412781.618	6205330.53	-1	0	-2	5
23	3350	412881.565	6205332.14	3	3	2	27
24	3808	412829.468	6205342.39	-2	4	0	5
25	1333	413080.476	6205344.37	3	20	0	4
26	3507	412852.505	6205345.07	0	0	0	6
27	5691	412685.349	6205357.13	-1	0	0	3
28	3178	412876.272	6205363.48	-3	0	-2	0
29	2134	412986.739	6205367.82	0	4	0	16
30	5496	412685.771	6205372.69	0	0	0	12
31	4728	412705.292	6205412.98	-1	1	-2	0
32	4285	412729.944	6205422.21	-3	0	-3	7
33	4458	412717.14	6205422.25	-2	0	-3	0
34	89	413135.255	6205437.21	3	17	0	23
35	663	413071.309	6205438.33	0	1	0	0
36	308	413107.182	6205440.7	3	3	3	16
37	1100	413014.2	6205454.89	-3	0	-2	6
38	2244	412905.211	6205456.51	-3	0	-3	1
39	585	413036.016	6205491.96	1	5	0	23
40	233	413067.13	6205501.06	-2	0	-3	4

4.3.1 Moisture Content

As discussed in Chapter 2, year-round maintenance of root-zone moisture is required to facilitate high levels of production of the black truffle. When rainfall does not exceed 1000mm annually, or is particularly low in summer months, irrigation is required to supplement moisture levels (Bradshaw, 2005). Soils with decent drainage are also essential to reduce the chance of host plants (such as Hazel and Oak) developing root rot (AgriFutures Australia, 2017). As the soil in Site B was sampled in late September after a period of relatively high winter rainfall (see Figure 4.1 Manjimup monthly mean rainfall (mm)), you would expect the soil to return high moisture content values (%) over the Site B study area.

Table 4.7 Lowest & highest soil moisture content (%) values and corresponding truffle yield for the Site B study area

Sample No.	Moisture Content (%) @ 105°C	Moisture Content (%) @ 40°C	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with lowest moisture content (%)						
20	20	16.6	0	4	2	13
22	20.9	18.2	-1	0	-2	5
24	21.4	18.2	-2	4	0	5
Samples with highest moisture content (%)						
33	32.6	30.4	-2	0	-3	0
11	32.5	29.9	0	0	-1	0
31	32.4	29.3	-1	1	-2	0
Average Moisture % at 105°C = 27.135 Average Moisture % at 40°C =24.555						

Table 4.7 suggests that the within the Site B study area, the soil was too moist to produce a high yield of truffles over the 2019 harvest season. In 2019, soil sample locations with lower moisture content (such as sample locations 20 and 22) returned little to no harvested truffles. Similarly, sample locations with high moisture content (such as sample locations 33 and 11) also produced low yields for the 2019 harvest season. In 2020, sample locations with high moisture content consistently produced no truffles while the sample location with the lowest moisture content (20) produced a relatively high number of truffles during the 2020 harvest season. This suggests that moister soils were not

conducive to high truffle yields in the 2020 harvest season, and that moisture content of 15% (@40°C) to 20% (@105°C) are capable of producing a high truffle yield, as long as the moisture content doesn't increase above 20% (measured @ 105°C). Figure 4.13 displays the soil moisture content profile for Site B and outlines how moisture content at 105°C varies across the study area in comparison to both the 2019 and 2020 truffle yield data. As the cold and hot spots for the 2019 and 2020 harvest years contain both high and low moisture contents, this suggests that soil moisture is not the most influential soil property affecting truffle yield within the Site B study area.

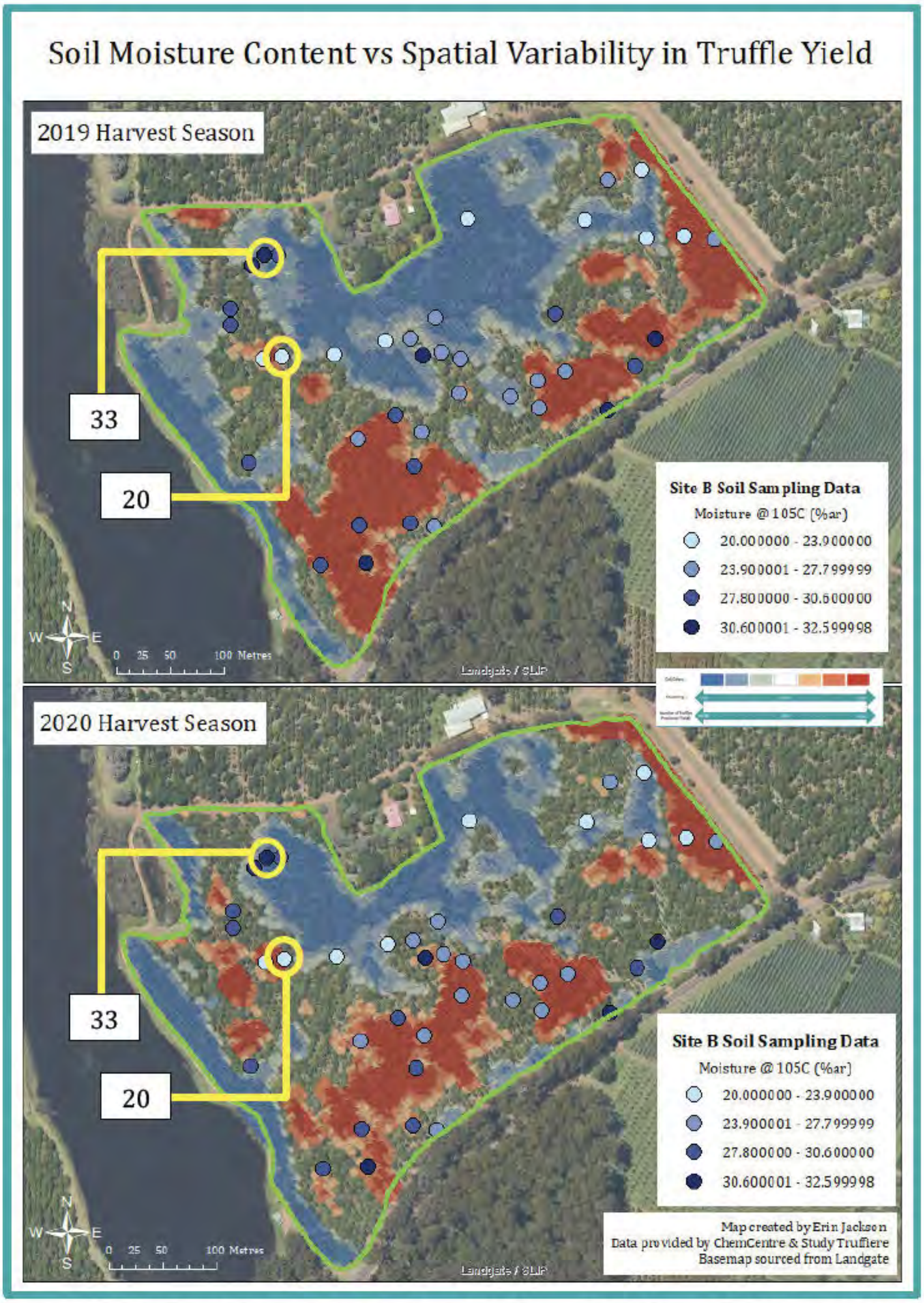


Figure 4.13 Site B – soil moisture content profile vs spatial variability in truffle yield

4.3.2 pH Levels

As stated in Chapter 2, for high yield of the black truffle, a pH between 7.2 and 8.3 is ideal. Generally speaking, soils that return a pH value of less that 6.5 can be considered acidic, and those that return a pH value over 7.5 are considered alkaline. pH values between 6.5 and 7.5 are considered to be neutral soils (Queensland Government (Environment Land & Water), 2013). Acidic soils can decrease the availability of essential plant nutrients, while also increasing the presence of toxic elements such as aluminium and manganese (Ball, 1999). Table 4.8 outlines the lowest and highest soil pH values for the Site B study area and their corresponding yield values for the 2019 and 2020 harvest seasons.

Table 4.8 Lowest & highest soil pH values and corresponding truffle yield for the Site B study area

Sample No.	pH Value	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
3	5.20 (lowest)	0	1	0	0
28	7.5 (equal highest)	-3	0	-2	0
13	7.5 (equal highest)	-1	0	0	20
Average pH value for Site B = 6.975					

Within the Site B study area soil pH averaged at approximately 6.975 (Table 4.7). The lowest pH (5.20) was measured at sample location three to the lower SW corner of the study area while the highest pH (7.5) was measured at both sample location 28 and 13 in the middle section of Site B (Figure 4.14). In the 2019 harvest season, all three of these sample locations returned low or no clustering with a low number of truffles harvested. Similarly, in the 2020 harvest season, sample locations with both low and high soil pH returned low harvest numbers (with the exception of sample location 13). Figure 4.14 displays the soil pH profile for Site B and outlines how pH values vary across the study area. This figure suggests that highly acidic soils (those samples returning pH values less than 6.5) generally produce little to no truffles over the studied harvest years. Neutral to more alkaline soils (pH values between 6.5 and 7.5) are capable of producing higher yields and are generally located in hot spots for the 2019 and 2020 harvest seasons. Those samples returning pH values (7.1 to 7.5) are almost always located in areas of high truffle yield. This suggest that alkaline soils are preferable in the production of the black truffle. In order for yield to improve in the future, the soil pH should be increased to the more ideal 7.9/8.3 pH range.

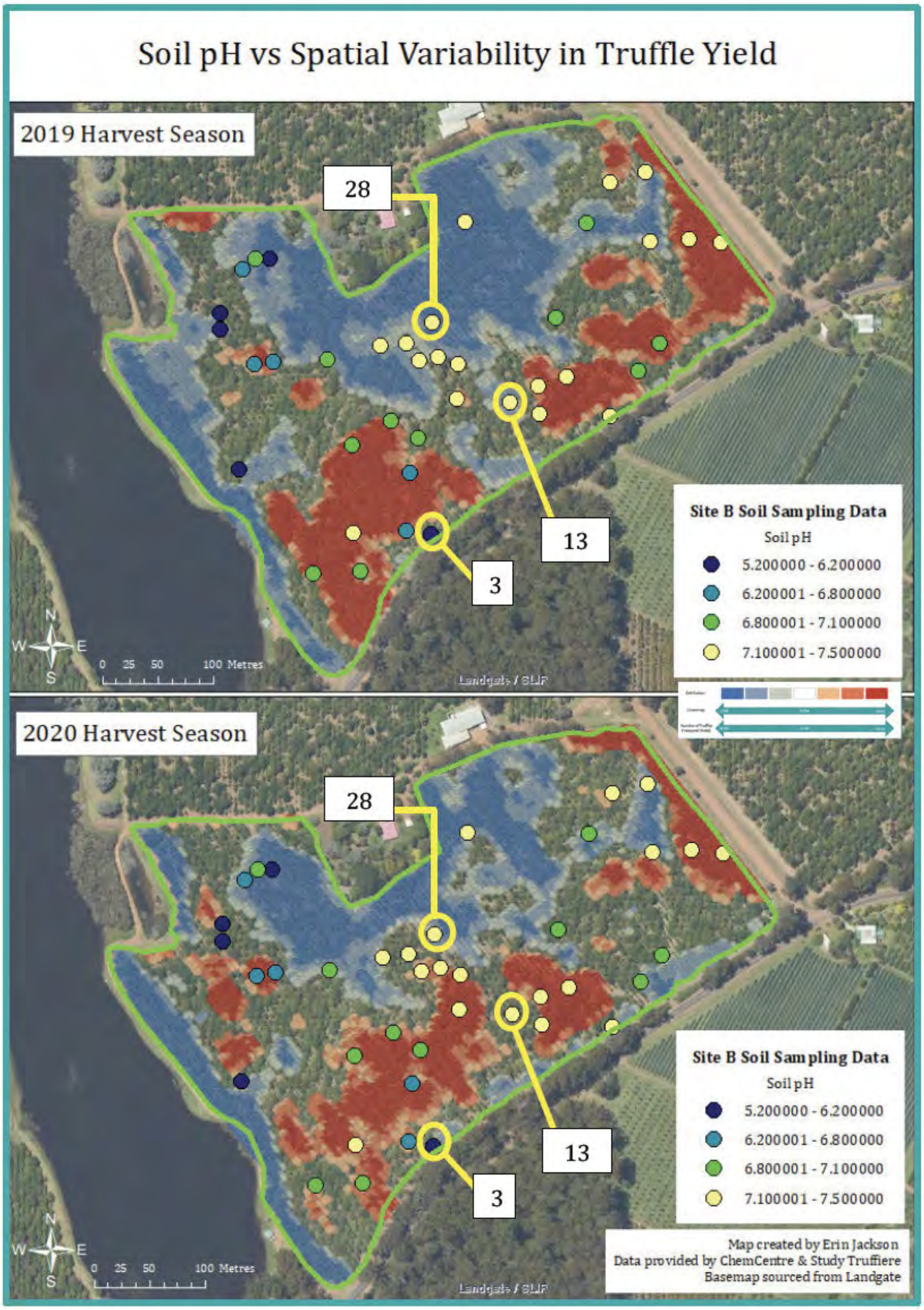


Figure 4.14 Site B –soil pH profile vs spatial variability in truffle yield

4.3.3 Soil Structure

As stated in Chapter 2, for high yield of the black truffle, soils must have a good aeriation and a loose structure to allow the developing truffles to grow (Castrignanò et al., 2000). Soils with decent drainage are also essential to reduce the chance of host plants (such as Hazel and Oak) developing root rot (AgriFutures Australia, 2017). Highly sandy soils drain quickly and remove the water from the roots systems, where soils with a high clay content may become waterlogged which impedes the growth and maturation of the black truffle. The ideal soil conditions best correlate to gravelly sandy loam to sandy clay loam surface soils. Figure 4.15 outlines how each soil sample taken within the Site B study area fit on the soil texture triangle.

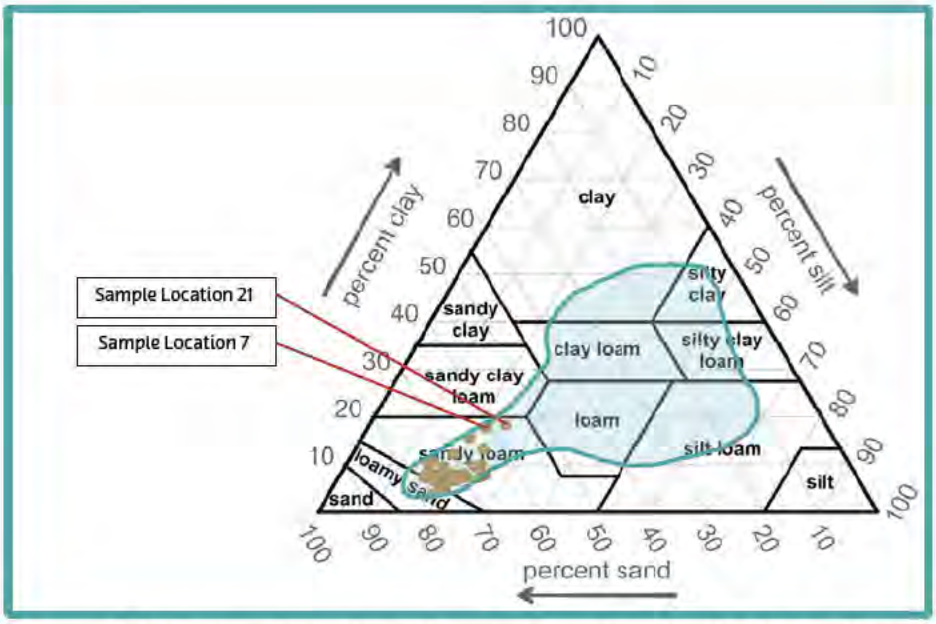


Figure 4.15 Texture triangle to show soil structure for the 40 Site B soil samples. Areas marked in blue are suitable for production of the black truffle

Figure 4.15 shows that all 40 soils samples fall into the loamy sand or sandy loam structure and are therefore suitable for the production of the black truffle. Table 4.9 illustrates that those samples that fall closest to the "ideal" sandy clay loam surface soil classification (sample locations 21 and 7) produce a higher yield of truffles. The soil structure ratios of sand/ silt/clay content for these samples are shown in Table 4.9.

Table 4.9 Ideal Sandy Clay Loam samples and their corresponding yield data

Sample No.	% Sand	% Silt	% Clay	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples closest to the ideal "sandy clay loam texture"							
21	60	21	19	-1	0	2	20
7	65	18	17	0	6	2	5

Tables 4.10 to 4.12 outline the samples with the highest and lowest sand, silt and clay content (%). Samples 3 and 12 contain both the highest sand content (80.5%) and the lowest clay content (6.5% and 5% respectively) where samples 21 and 7 contain the lowest sand content (60% and 65%) and the highest clay content (19% and 17% respectively) over the Site B study area. Based on Figure 4.16 which outlines how soil sand, silt and clay content varies across the study area in comparison to both the 2019 and 2020 truffle yield data, generally speaking, soils with 76.5%-80.5% sand content produce a higher number of truffles than those containing 60%-65% sand. Soils with a lower percentage of silt (12%) and clay (5% - 7.5%) tend to be correlated with areas of higher truffle yield.

Tables 4.10 to 4.12 and Figure 4.16 suggest the soils within the Site B study area do not highly influence truffle yield over the 2019 and 2020 harvest seasons. High and low sand, silt and clay contents are all capable of producing high and low truffle yield during the studied years. This suggests that soil structure within Site B does not vary to the point in which it begins to affect truffle yield. As all 40 soils samples fall into the loamy sand or sandy loam structure, Site B is therefore suitable for the production of the black truffle.

Table 4.10 Lowest & highest sand content and corresponding truffle yield for the Site B study area

Sample No.	% Sand	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest sand content (%)					
3	80.5	0	1	0	0
12	80.5	1	2	1	12
Samples with lowest sand content (%)					
21	60	-1	0	2	20
7	65	0	6	2	5
Average sand content (%) for all 40 samples: 74%					

Table 4.11 Lowest & highest silt content and corresponding truffle yield for the Site B study area

Sample No.	% Silt	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest silt content (%)					
30	23	0	0	0	12
31	22	-1	1	-2	0
Samples with lowest silt content (%)					
36	12	3	3	3	16
40	13	-2	0	-3	4
Average silt content (%) for all 40 samples: 17%					

Table 4.12 Lowest & highest clay content and corresponding truffle yield for the Site B study area

Sample No.	% Clay	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest clay content (%)					
21	19	-1	0	2	20
7	17	0	6	2	5
37	16	-3	0	-3	1
Samples with lowest clay content (%)					
12	5	1	2	1	12
20	6.5	0	4	2	13
3	6.5	0	1	0	0
5	6.5	3	4	0	9
Average clay content (%) for all 40 samples: 8%					

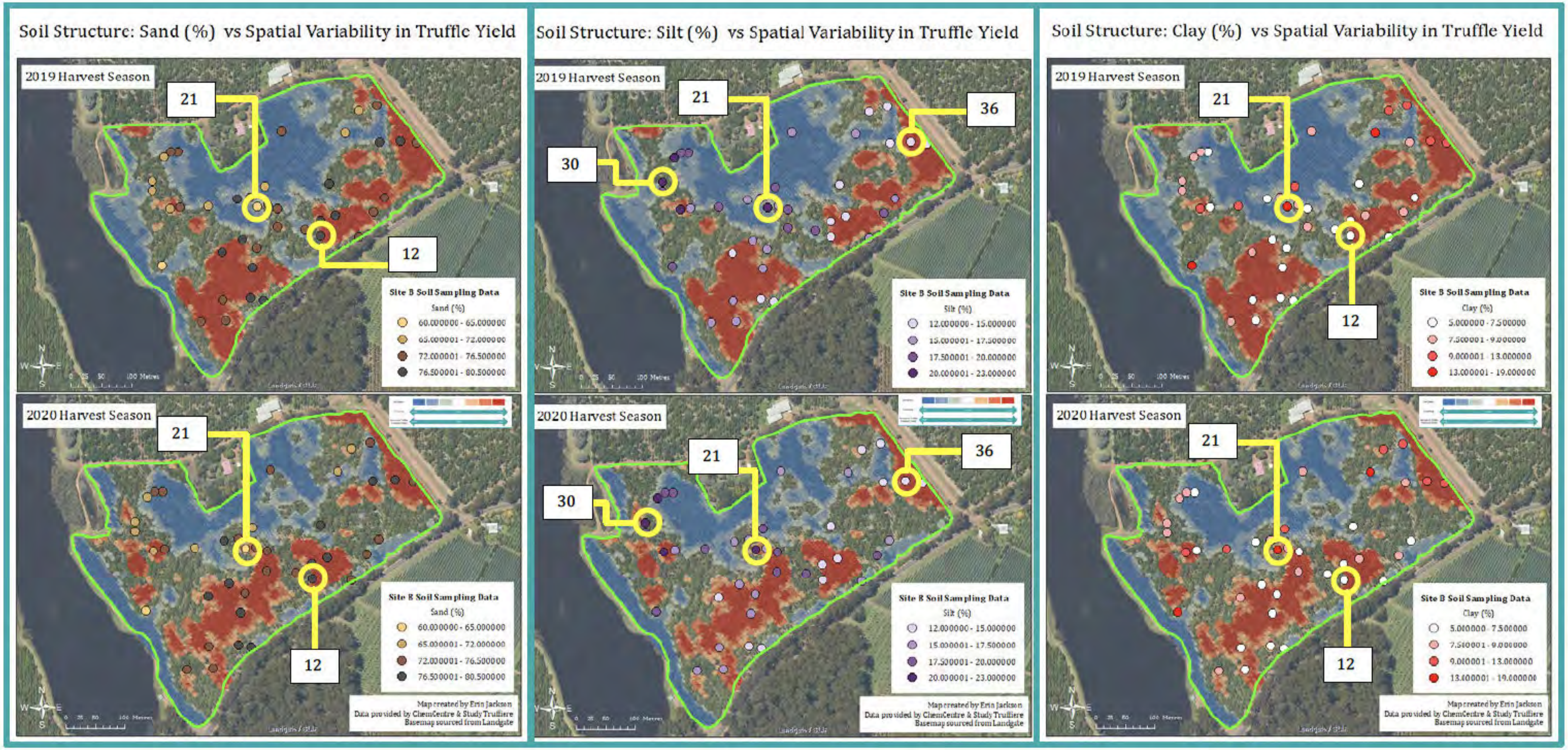


Figure 4.16 Site B – soil structure profile vs spatial variability in truffle yield

4.3.4 Nutrient Ratios

As stated in Chapter 2, a specific nutrient ratio is required for high production of the black truffle. The black truffle ideally grows in soils with relatively balanced ratio of soil nutrients such as *"phosphorus, calcium, magnesium, nitrogen, potassium, sulphur and trace elements of boron, copper, iron, manganese and zinc"* (Hall et al., 2007). The most productive truffières require high concentrations of plant available calcium (which due to limitations involved in measuring this soil nutrient, will not be used in this study) and magnesium, as well as moderate levels of phosphorus and little concentration of sodium. Research shows that truffières are rarely productive in soils with an unbalanced ratio of phosphorus to calcium and potassium (Hall et al., 2007). Acidic soils, like those found in the Site B study area increase the presence of toxic elements such as aluminium and manganese. Tables 4.13 to 4.23 outline the sample location with the lowest and highest content of each soil nutrient, and their corresponding truffle yield for the Site B study area.

4.3.4.1 Trace Nutrients - Boron, Copper, Iron, Manganese & Zinc

Table 4.13 demonstrates that sample locations containing both high (2.3mg/kg) and low (0.7mg/kg) concentrations of boron are capable of producing the black truffle. This suggests that trace concentrations of boron (between 0.5mg/kg and 2.3mg/kg) within the soils in the Site B study area do not highly influence yield results during the 2019 and 2020 harvest seasons.

Table 4.13 Lowest & highest boron content and corresponding truffle yield for the Site B study area

Sample No.	Boron content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Boron (mg/kg)					
13	2.3	-1	0	0	20
14	1.6	2	3	3	25
11	1.6	0	0	-1	0
Samples with lowest Boron (mg/kg)					

3	0.5	0	1	0	0
7	0.7	0	6	2	5
32	0.7	-3	0	-3	7
30	0.7	0	0	0	12
Average Boron (mg/kg) for all 40 samples: 1.15mg/kg					

Table 4.14 suggests that within the Site B study area sample locations in which soils contain both high (5.1mg/kg) and low (0.4mg/kg) copper are capable of producing high yields of truffle over the 2019 and 2020 harvest seasons. As copper is a trace element in the production of the black truffle, less than 5.1mg/kg of copper within the soil is essential to maintain high yield results with "*high copper concentrations [having] detrimental effect on root growth*" (Hall et al., 2007, p. 182). When copper concentration increases above 5.1mg/kg (e.g., sample location 32), yield rapidly declines. This theory is further shown in Figure 4.17 *Site B soil copper content (mg/kg) vs spatial variability in truffle yield* where sample locations containing lower soil copper contents are largely found in areas of high truffle yield (hot spots) and sample locations with higher soil copper contents (especially sample location 32) are located in areas of lower truffle yield (cold spots) over the 2019 and 2020 harvest seasons.

Table 4.14 Lowest & highest copper content and corresponding truffle yield for the Site B study area

Sample No.	Copper content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Copper (mg/kg)					
32	6.3	-3	0	-3	7
14	5.1	2	3	3	25
Samples with lowest Copper (mg/kg)					
36	0.4	3	3	3	16
34	0.4	3	17	0	23
Average Copper (mg/kg) for all 40 samples: 1.83mg/kg					

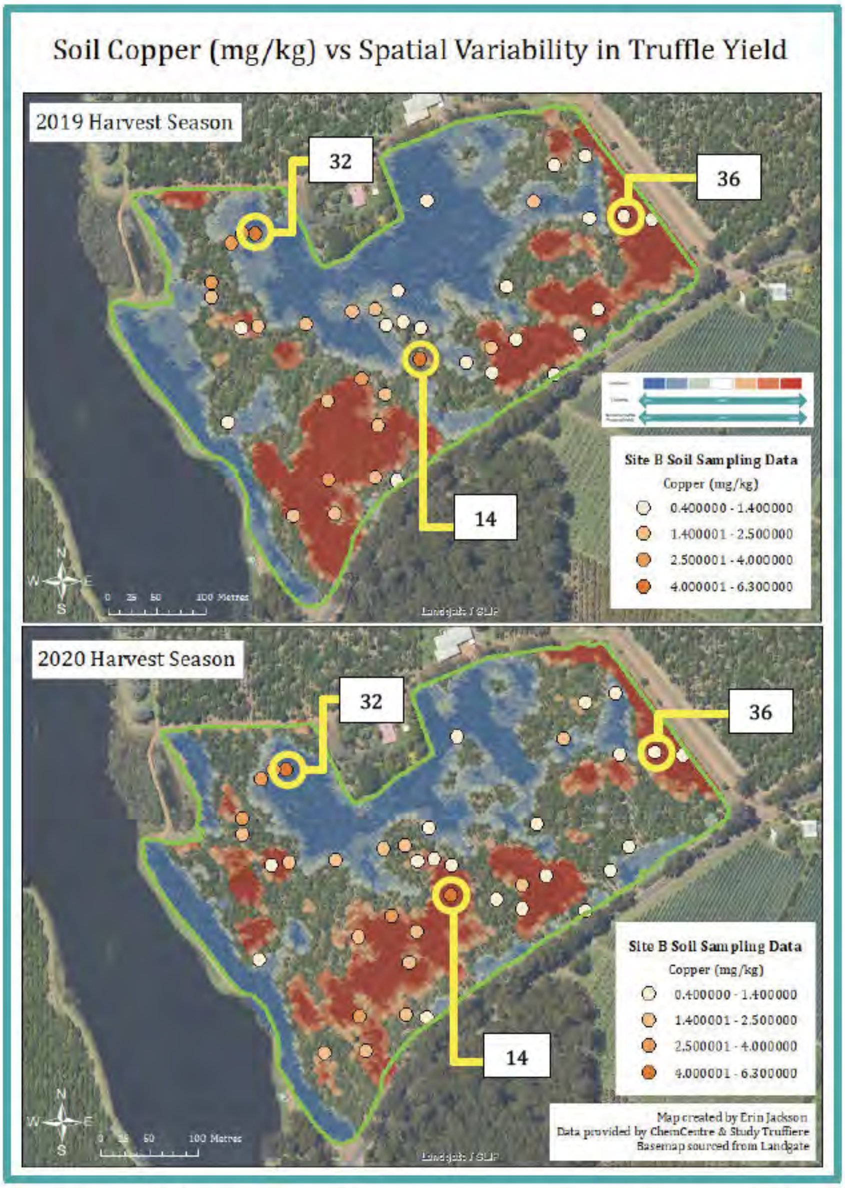


Figure 4.17 Site B – soil copper content (mg/kg) vs spatial variability in truffle yield

Table 4.15 outlines the sample locations within the Site B study area with the lowest and highest iron content, and their corresponding truffle yield for the 2019 and 2020 harvest seasons. As with boron and copper, iron is a trace element in the production of the black truffle. Table 4.15 illustrates that both high and low soil iron contents are capable of producing a high truffle yield. Soils within the Site B study area vary in iron content between 31mg/kg (sample location 25) and 78mg/kg (sample location 7). Varying iron content within the soil across the study area is therefore not significant enough to highly influence yield numbers over the 2019 and 2020 harvest seasons.

Table 4.15 Lowest & highest iron content and corresponding truffle yield for the Site B study area

Sample No.	Iron content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Iron (mg/kg)					
7	78	0	6	2	5
20	72	0	4	2	13
Samples with lowest Iron (mg/kg)					
25	31	3	20	0	4
12	32	1	2	1	12
Average Iron (mg/kg) for all 40 samples: 46.8mg/kg					

As discussed, toxic elements such as manganese can increase their presence in soils with increasing soil acidity. Table 4.16 suggests that the soils in the Site B study area do not contain enough manganese to toxify the soil and impact the ability for truffles to grow. Both high and low manganese soil contents are capable of producing high and low truffle yields over the 2019 and 2020 harvest seasons. Additionally, there seems to be no spatial pattern linking the distribution of the manganese content and truffle yield in the studied harvest years. Similar trends are evident when analysing the zinc content (mg/kg) of the soils in the Site B study area (Table 4.17).

Table 4.16 Lowest & highest manganese content and corresponding truffle yield for the Site B study area

Sample No.	Manganese content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Manganese (mg/kg)					
7	13	0	6	2	5
21	10	-1	0	2	20
Samples with lowest Manganese (mg/kg)					
39	3.7	1	5	0	23
29	4	0	4	0	16
Average Manganese (mg/kg) for all 40 samples: 6.59mg/kg					

Table 4.17 Lowest & highest zinc content and corresponding truffle yield for the Site B study area

Sample No.	Zinc content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Zinc (mg/kg)					
39	3.6	1	5	0	23
32	2.8	-3	0	-3	7
Samples with lowest Zinc (mg/kg)					
3	0.4	0	1	0	0
7	0.5	0	6	2	5
Average Zinc (mg/kg) for all 40 samples: 1.43mg/kg					

4.3.4.2 Main Nutrients - Magnesium, Nitrogen, Phosphorous, Potassium, Sodium & Sulphur

Table 4.18 outlines the soil sample locations with the highest and lowest magnesium contents, as well as their corresponding truffle yield for the 2019 and 2020 harvest season. Both high (670mg/kg at sample location 13) and low (150mg/kg at sample location 3) magnesium content are capable of producing high and low truffle yield. As the largest variation in truffle yield occurs from season to season, rather than low and high magnesium soil content, it is unlikely that the concentration of this element within the soil influences truffle yield within the Site B study area.

Table 4.18 Lowest & highest magnesium content and corresponding truffle yield for the Site B study area

Sample No.	Magnesium content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Magnesium (mg/kg)					
13	670	-1	0	0	20
21	580	-1	0	2	20
Samples with lowest Magnesium (mg/kg)					
3	150	0	1	0	0
36	250	3	3	3	16
Average Magnesium (mg/kg) for all 40 samples: 417mg/kg					

Table 4.19 suggests that there is a strong relationship between the concentration of nitrogen within the soil in the Site B study area, and the corresponding truffle yield. Soils that contain higher nitrogen contents, such as sample location 33 (0.88% nitrogen), consistently produce little to no truffles over the 2019 and 2020 harvest seasons. Soils with low nitrogen contents, such as sample location 36 (0.25% nitrogen), consistently produce a high yield of the black truffle over the studied years. This trend is further confirmed in Figure 4.18 *Site B soil nitrogen content (%) vs spatial variability in truffle yield* in which areas of high truffle yield (hot spots) are spatially correlated with those samples returning lower nitrogen content, and samples with higher nitrogen content are spatially correlated with areas of low truffle yield (cold spots).

Table 4.19 Lowest & highest nitrogen content and corresponding truffle yield for the Site B study area

Sample No.	Nitrogen content (%)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Nitrogen (%)					
33	0.88	-2	0	-3	0
Samples with lowest Nitrogen (%)					
36	0.25	3	3	3	16
Average Nitrogen for all 40 samples: 0.51%					

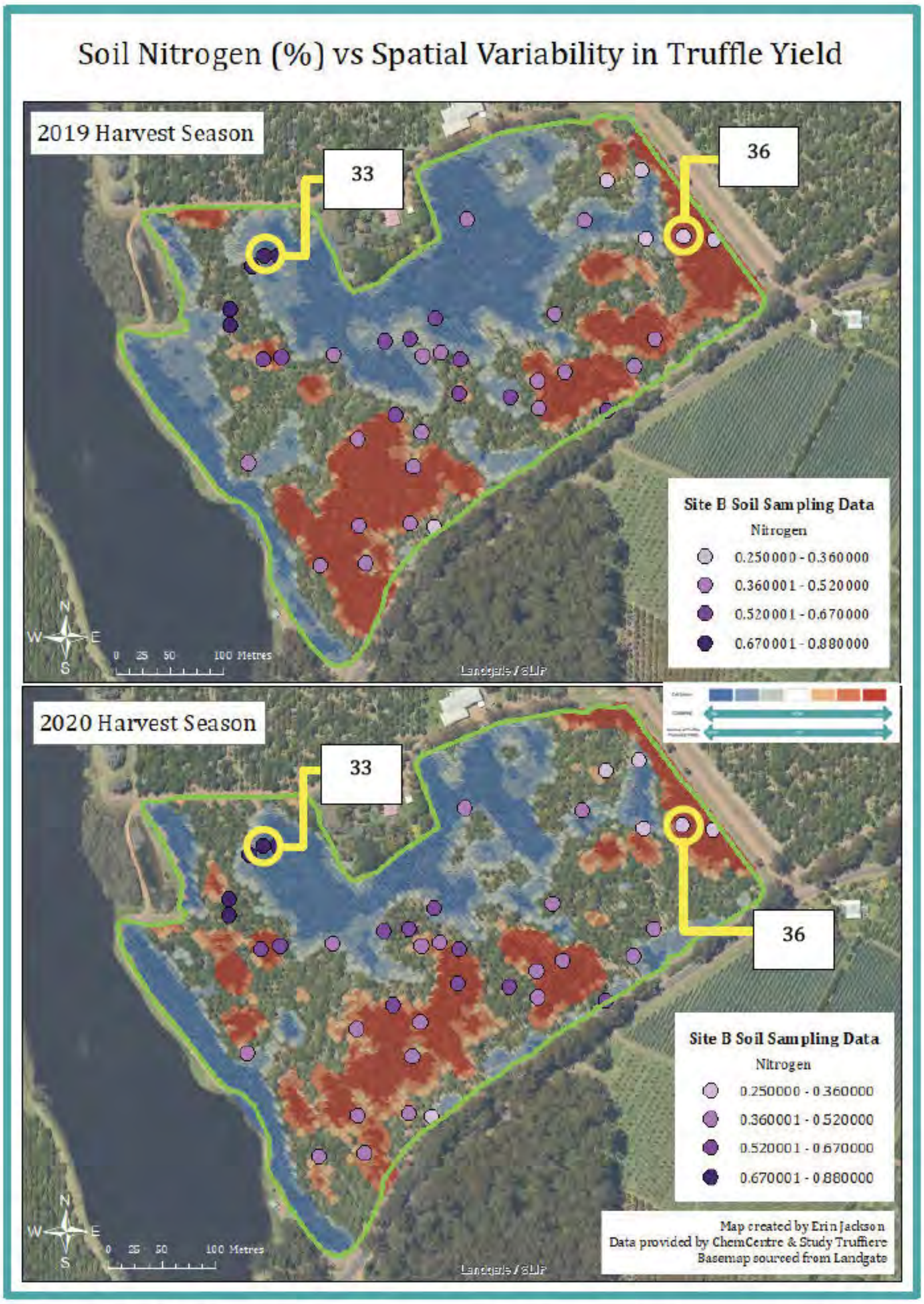


Figure 4.18 Site B – soil nitrogen content (%) vs spatial variability in truffle yield

Table 4.20 suggests that the concentration of phosphorous within the Site B study area soils are unlikely to have a high influence over truffle yield. High phosphorous concentrations, like those found in sample locations 20 (51mg/kg) and 22 (51mg/kg) produced both high and low truffle yield in the 2020 harvest season. Similarly, those sample locations with low concentrations of phosphorous, such as sample locations 17 (7mg/kg) and 11/16 (both 9mg/kg) produced little to no truffles in the 2019 harvest season, and a large number of truffles in the 2020 harvest season. This suggests the phosphorous concentrations in the soils within the Site B study area are not low or high enough to have any influence over yield results in the studied harvest periods.

Table 4.20 Lowest & highest phosphorous content and corresponding truffle yield for the Site B study area

Sample No.	Phosphorous content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Phosphorous (mg/kg)					
20	56	0	4	2	13
22	51	-1	0	-2	5
Samples with lowest Phosphorous (mg/kg)					
17	7	0	9	-1	2
11	9	0	0	-1	0
16	9	3	6	3	16
Average Phosphorous (mg/kg) for all 40 samples: 20mg/kg					

Table 4.21 outlines the sample locations with the lowest (40mg/kg) and highest (210mg/kg) soil potassium concentrations within the Site B study area. This table suggests that those samples with a higher concentration of potassium, such as sample locations 4 & 5 are correlated with high truffle yield, whereas those samples with low concentrations of potassium (sample locations 35 and 40), consistently return little to no truffles for the 2019 and 2020 harvest seasons. This theory is further reinforced in Figure 4.19 *Site B soil potassium content (mg/kg) vs spatial variability in truffle yield.* Areas of high truffle yield (hot spots) are generally correlated with sample locations that return a higher potassium content. Those samples with lower potassium content tend to be located in (with a few exceptions in the NW section of Site B) areas of lower truffle yield (cold spots) for the 2019 and 2020 harvest seasons.

Table 4.21 Lowest & highest potassium content and corresponding truffle yield for the Site B study area

Sample No.	Potassium content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Potassium (mg/kg)					
4	210	3	5	3	9
5	210	3	4	0	9
Samples with lowest Potassium (mg/kg)					
40	40	-2	0	-3	4
35	40	0	1	0	0
Average Potassium (mg/kg) for all 40 samples: 117.98m/.kg					

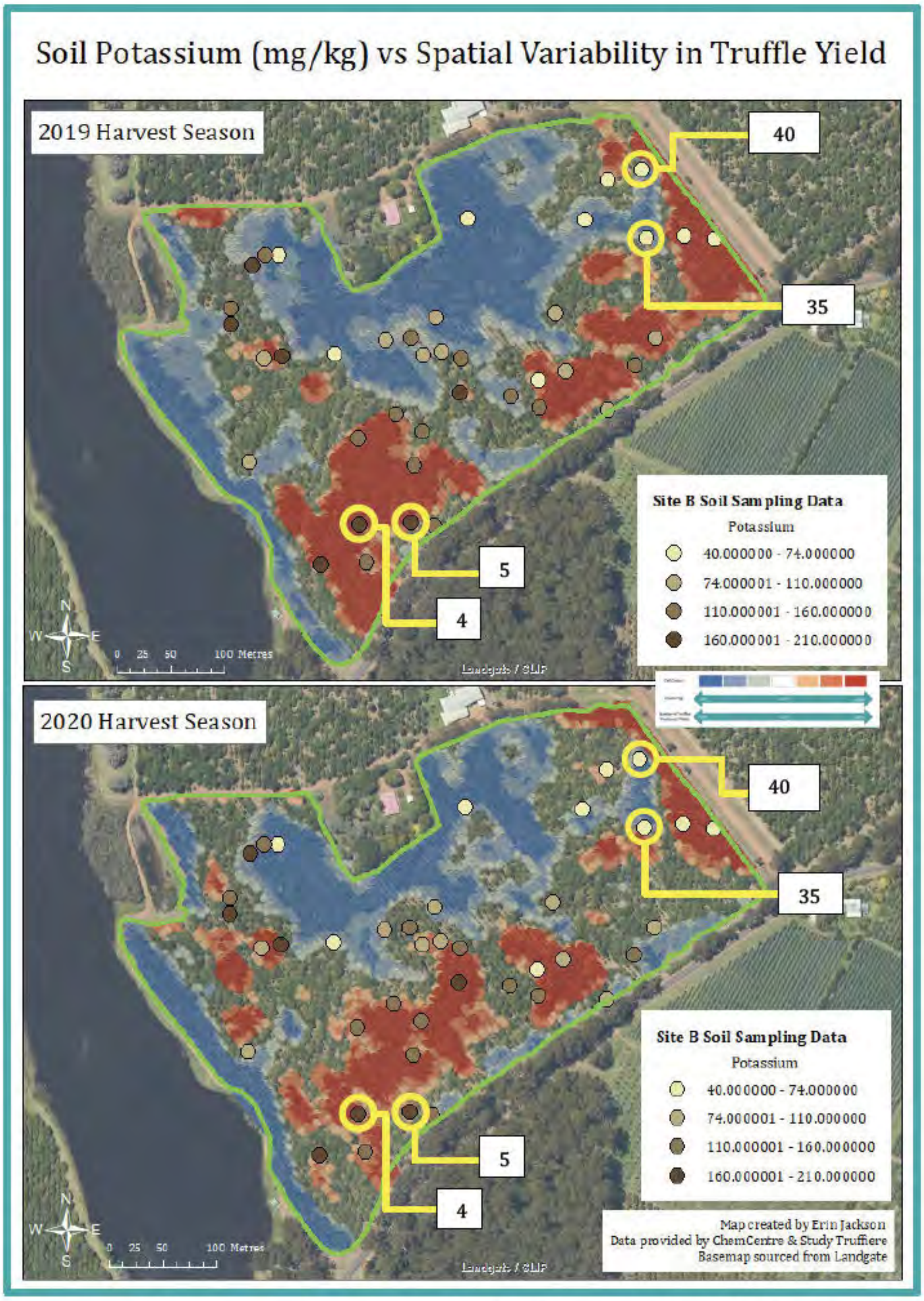


Figure 4.19 Site B – soil potassium (mg/kg) vs spatial variability in truffle yield

Table 4.22 suggests that like many other of the nutrients studied, varying levels of sodium within the soils in the Site B study area has little influence over yield for the 2019 and 2020 harvest seasons. Both sample locations with high sodium content (79mg/kg at sample location 14, and 76mg/kg) were capable of producing high and low truffle yield. Similarly, sample locations 37 and 36 measured low sodium content (at 31mg/kg and 34mg/kg) and produced a high and low yield of truffles over the studied harvest periods. Additionally, there is no clear spatial pattern between sodium content and truffle yield for the 2019 and 2020 harvest seasons that would suggest a relationship exists between these two properties. Sodium concentrations measured at these sample locations must therefore not be high or low enough to affect yield results.

Table 4.22 Lowest & highest sodium content and corresponding truffle yield for the Site B study area

Sample No.	Sodium content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Sodium (mg/kg)					
14	79	2	3	3	25
31	76	-1	1	-2	0
Samples with lowest Sodium (mg/kg)					
37	31	-3	0	-2	6
36	34	3	3	3	16
Average Sodium (mg/kg) for all 40 samples: 54.7mg/kg					

Table 4.23 outlines the sample locations with the lowest (9mg/kg) and highest (20mg/kg) soil sulphur concentrations within the Site B study area. Table 4.23 suggests that soils with high sulphur content, such as sample locations 24 and 13 are not conducive to a high truffle yield. Both these samples returned a sulphur content of 20mg/kg and produced little to no truffles over the 2019 and 2020 harvest seasons. Those sample locations that returned low sulphur contents such as 12 and 16 (10mg/kg) yielded a high number of truffles in both the 2019 and 2020 harvest season. This trend is further shown in Figure 4.20. Areas of high truffle yield (hot spots) are generally correlated with sample locations that return lower (9kg/kg 12mg/kg) sulphur contents. Those samples with higher sulphur

content (18mg/kg 20mg/kg) tend to be located in areas of lower truffle yield (cold spots) for the studied harvest seasons.

Table 4.23 Lowest & highest sulphur content and corresponding truffle yield for the Site B study area

Sample No.	Sulphur content (mg/kg)	2019 Density OHS	2019 Yield	2020 Density OHS	2020 Yield
Samples with highest Sulphur (mg/kg)					
24	20	-2	4	0	5
13	20	-1	0	0	20
Samples with lowest Sulphur (mg/kg)					
37	9	-3	0	-2	6
12	10	1	2	1	12
16	10	3	6	3	16
Average Sulphur (mg/kg) for all 40 samples: 14 25mg/kg					

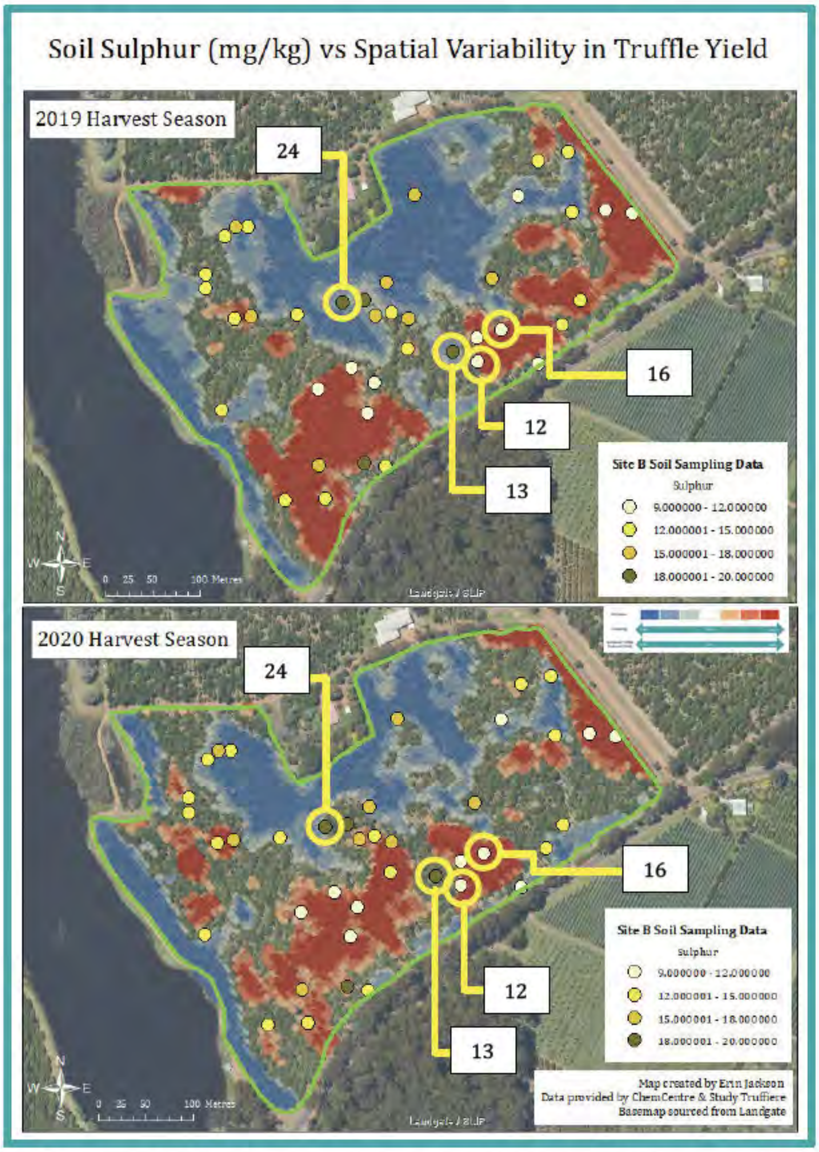


Figure 4.20 Site B - soil sulphur (mg/kg) vs spatial variability in truffle yield

4. 4 Regression Analysis

So far, this chapter has suggested that certain soil properties within the Site B study area may have influenced truffle yield over the 2019 and 2021 harvest seasons. The soil profile created for Site B suggested that soils were generally too moist to facilitate a high level of truffle production, with the highest yield occurring at sample location 20, which returned the lowest moisture content at 15% (at 40°c) to 20% (at 105°C). Highly acidic soils in Site B (those samples returning pH values less than 6.5) generally produced little to no truffles over the studied harvest years. Sample locations with neutral soils (pH values between 6.5 and 7.5) were capable of producing higher yields and were generally located in hot spots for the 2019 and 2020 harvest seasons. Those samples returning higher pH values (7.1 to 7.5) were almost always located in areas of high truffle yield. This suggests that alkaline soils are preferable in the production of the black truffle. Within Site B, the soil structure (the percentage makeup of sand, silt and clay) did not vary significantly to which it began to affect truffle yield. All 40 soils samples fell into the loamy sand or sandy loam structure were therefore suitable for the production of the black truffle.

Traces elements including boron, iron, manganese and zinc seemed to have minimum influence over yield in the 2019 and 2020 harvest seasons. In order to facilitate higher truffle yields, it is essential for soils within Site B to contain less than 5.1mg/kg of copper. When copper content increased above 5.1mg/kg (sample location 32), truffle yield rapidly declined. Sample locations containing higher nitrogen and sulphur contents consistently produce little to no truffles where sample locations with low nitrogen and sulphur contents consistently produce a high yield of the black truffle over the studied years. A weak relationship was also evident between truffle yield in the studied seasons, and the potassium content within the soil. Soils with higher concentrations of potassium were correlated with areas of high truffle yield, whereas samples with low concentrations of potassium consistently return little to no truffles in the studied harvest years. Elements such as magnesium, phosphorous and sodium visually had little influence over yield for the 2019 and 2020 harvest seasons.

This section of the analysis will aim to statistically confirm whether the soils properties identified above (soil moisture, pH and copper, nitrogen, sulphur and potassium contents) influence truffle yield over the 2019 and 2020 harvest seasons. The *Modelling Spatial Relationships* toolset within ArcGIS contains tools which determine the variables (e.g., soil properties) that explain why an observation pattern (e.g., variation in truffle yield) is present (Pimpler, 2017). Regression analysis aims to solve the following regression equation;

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \dots \dots \beta_n X_n + \varepsilon$$

Where:

y is the dependent variable (truffle yield in 2019 and 2020);

β_0, β_1 & β_2 are the regression coefficients (values that are computed by the regression tool that represents strength a type (positive or negative) or the relationship);

X_1, X_2 are the explanatory variables (the soil properties), and;

ε is the random error term / residual (the "unexplained" portion of the dependent variable) (Pimpler, 2017).

The regression equation also calculates p-values and R^2 values for each coefficient. P-values are measures of probability where small values suggest that the variable is important to the model (Pimpler, 2017). For example, a p-value of 0.01 indicates that there is a 99% probability that the coefficient is statistically significant and therefore the associated variable could be used as an effective predictor of the dependent variable (Environmental Systems Research Institute (ESRI), 2022). R^2 values range from 0 to 100% and quantify the model performance. A perfect model will return a R^2 value of 1.0, though this rarely occurs in practice. Within this study, the adjusted R^2 value will be used as it is considered to be more accurate when considering multiple variables (Environmental Systems Research Institute (ESRI), 2022).

Exploratory Regression tool is a good starting point for analysis and will help evaluate which soil properties **best** explain the number of harvested truffles within the Site B study area in both the 2019 and 2020 harvest seasons. This information will then be used within the *Ordinary Least Squares (OLS)* tool to determine the linear relationship between the dependent variable (truffle yield) and the exploratory variables (soil properties). This tool will help identify whether a positive or negative relationship exists between a given soil property and the number of harvested truffles; and provide some insights to the strength of these relationships.

4.4.1 Exploratory Regression

In order to complete the regression analysis, a new dataset containing each sample location and it's corresponding soil properties and truffle yield for the 2019 and 2020 harvest seasons was created. The *Exploratory Regression* tool was then run on this dataset, using the 2019 and 2020 truffle yields as the dependent variables, and the chosen soil properties (moisture at 105°C, pH, nitrogen, copper, sulphur and potassium contents) as the candidate explanatory variables. The maximum number of explanatory variables was set to 6, and the minimum acceptable adjusted R squared value was set to 0.25 (in which a given soil property has to explain at least 25% of the variation in truffle yield to be considered significant). All other search criteria were left as the default values. This tool models the relationship between an exploratory variable (e.g., soil pH) or set of variables (e.g., soil pH and soil moisture) to the dependent variable (truffle yield) and lists the three models with the highest adjusted R^2 value. Within this analysis we will only be looking at the section that compares one explanatory variable to the dependant variable at a time. 4.24 outlines the results of the *Exploratory Regression* tool.

Table 4.24 Exploratory Regression - 2019 & 2020 Harvest Seasons

Exploratory Regression - 2020 Harvest Yield											
Soil Property	Adjusted R^2 value	Akaike's Information Criterion (AICc)	Jarque-Bera p-value (JB)	Koenker (BP) Statistic p-value (K(BP))	Max Variance Inflation Factor (VIF)	Global Moran's I p-value (SA)	Model Relationship	Model Significance (* = 0.10; ** = 0.05; ***=0.01)	Significance (%)	Negative (%)	Positive (%)
Moisture (%)	0.01	301.53	0.00	0.12	1.00	0.55	negative	-	0.00	100.00	0.00
pH	0.01	301.43	0.00	0.93	1.00	0.53	positive	-	0.00	0.00	100.00
Nitrogen (%)	-0.01	302.28	0.00	0.81	2.00	0.69	negative	-	0.00	93.75	6.25
Copper (mg/kg)	-	-	-	-	-	-	-	-	0.00	0.00	100.00
Sulphur (mg/kg)	-	-	-	-	-	-	-	-	0.00	9.38	90.62
Potassium (mg/kg)	-	-	-	-	-	-	-	-	0.00	96.88	3.12
Exploratory Regression - 2019 Harvest Yield											
Soil Property	Adjusted R^2 value	Akaike's Information Criterion (AICc)	Jarque-Bera p-value (JB)	Koenker (BP) Statistic p-value (K(BP))	Max Variance Inflation Factor (VIF)	Global Moran's I p-value (SA)	Model Relationship	Model Significance (* = 0.10; ** = 0.05; ***=0.01)	Significance (%)	Negative (%)	Positive (%)
Moisture (%)	-	-	-	-	-	-	-	-	0.00	0.00	100.00
pH	-	-	-	-	-	-	-	-	0.00	40.62	59.38
Nitrogen (%)	0.05	243.07	0.00	0.27	1.00	0.84	negative	*	9.38	100.00	0.00
Copper (mg/kg)	0.04	243.46	0.00	0.08	1.00	0.94	negative	**	3.12	100.00	0.00
Sulphur (mg/kg)	-0.01	245.39	0.00	0.53	1.00	0.75	negative	-	0.00	100.00	0.00
Potassium (mg/kg)	-	-	-	-	-	-	-	-	0.00	15.62	84.38

Table 4.24 confirms the theory that over the 2019 and 2020 harvest season, soil moisture, pH, nitrogen, copper and sulphur contents all had some statistically significant influence over truffle yield. The low Jarque-Bera values for both years indicates that there may be some bias present within the models and suggests that the dataset may contain some data outliers, or the relationships between a soil property and truffle yield may be non-linear. Although the soil property variables didn't necessarily pass the 0.25 R^2 requirement, both the 2019 and 2020 regressions passed the maximum VIF value and minimum spatial autocorrelation p-value tests. The 2019 and 2020 *Exploratory Regression* results will therefore be used to determine which soil properties should be analysed in greater detail using the *Ordinary Least Squares* tool.

Within the 2020 harvest year, both soil pH and soil moisture returned the highest adjusted R^2 result, both with a value of 0.01, and nitrogen with a value of -0.01. The R^2 values outline how well the exploratory variable is predicating the dependent variables value. These values above confirm that some relationship does exist between truffle yield and the sample locations corresponding soil pH, soil moisture and nitrogen content values. Additionally, Table 4.24 confirms that a positive linear relationship exists between truffle yield and soil pH (i.e. as soil pH increase, truffle yield is also likely to increase) and a negative linear relationship exists between truffle yield and soil moisture and nitrogen content (as moisture or nitrogen content levels increase, truffle yield decreases).

In the 2019 harvest season, nitrogen content (%) returned the highest adjusted R^2 result, with a value of 0.05, followed by copper content (mg/kg) with a R^2 value of 0.04 and finally sulphur content with a R^2 value of -0.01. All 3 of these soil properties returned a negative linear relationship, confirming the theory that as nitrogen, copper and sulphur increases in the soil, truffle yield decreases. Nitrogen and copper content both returned some level of model significance, returning a significance value of 9.48% (significance p-value of 0.10) and 3.13% (significance p-value of 0.05) indicating that they are fairly strong predictors of truffle yield. For both the 2019 and 2020 harvest years, the regression results found that soil potassium content is not statistically affecting truffle yield within the Site B study area.

4.4.2 Ordinary Least Squares

The *Ordinary Least Squares* tool was run on the dataset containing each sample location and their corresponding soil properties and truffle yield for the 2019 and 2020 harvest seasons. The yield data for 2019 and 2020 were used as the dependent variables and the statistically significant soil properties identified above were used as the explanatory variables. For the 2020 harvest season, these soil properties include soil moisture content (%), pH and nitrogen content (%). For the 2019 harvest season, the selected soil properties include nitrogen content (%), copper content (mg/kg) and soil content (%). The *Ordinary Least Squares* for the studied harvest seasons are shown below (Table 4.25)

Table 4.25 Ordinary Least Squares – 2019 & 2020 Harvest Seasons

Ordinary Least Squares - 2020 Harvest Yield									
Soil Property	Regression Coefficient	Relationship	Standard Error	Probability	Robust Probability	Koenker (BP) Statistic	Jarque - Bera Statistic	Multiple R^2 Value	Adjusted R^2 Value
Moisture (%)	-0.534	negative	0.460	0.2532	0.2557	2.4797	18.534	0.0342	0.0088
pH	4.034	positive	3.357	0.2368	0.0910	0.00742	42.044	0.0366	0.0113
Nitrogen (%)	-7.605	negative	10.51	0.4737	0.3748	0.0601	34.933	0.0136	-0.0124
Ordinary Least Squares - 2019 Harvest Yield									
Soil Property	Regression Coefficient	Relationship	Standard Error	Probability	Robust Probability	Koenker (BP) Statistic	Jarque - Bera Statistic	Multiple R^2 Value	Adjusted R^2 Value
Nitrogen (%)	-8.631	negative	5.01	0.0929	0.0289*	1.198	34.61	0.0725	0.0481
Copper (mg/kg)	-0.982	negative	0.612	0.1167	0.0497*	3.066	22.48	0.064	0.0389
Sulphur (mg/kg)	-0.219	negative	0.270	0.4222	0.27045	0.3871	34.23	0.017	-0.009

The *Ordinary Least Squares* tool confirms that a strong negative relationship exists between truffle yield and soil nitrogen content (%) in both the 2019 and 2020 harvest seasons. Additionally, a strong negative relationship exists between truffle yield and soil copper content (mg/kg) and sulphur content (mg/kg) in the 2019 harvest season, and truffle yield and soil moisture (%) in the 2020 harvest season. A strong positive relationship also exists between soil pH and truffles harvested in the 2020 season.

Both nitrogen and copper content returned somewhat statistically significant results in 2019, as indicated by the asterisk (*) next to the calculated robust probability. This suggests that within the 2019 harvest season, the content of nitrogen (%) and copper (%) played an important role in the variability of truffles over the Site B study area. No single property within the 2020 harvest season proved to be statistically significant. Although the other measured soil properties did not pass this check of statistical significance, it does not necessarily mean they don't also play a part in the distribution of truffles over the studied harvest years. In order to determine the extent of which soil property / properties have the most influence over yield, a soil scientist with a greater understanding of soil chemistry is required to further analyse these results. Additionally, a soil scientist may assist in identifying whether it is a combination of soil properties, rather than just a single one, that work in symbiosis with each other to produce a higher yield of truffles in the Site B study area.

4. 5 Chapter Summary

This chapter presented the findings and analysis of this research and outlined how the collected data was processed in order to produce the research results. For a truffière to produce a high yield of truffles, they must experience warm summer temperature, to initiate the truffle formation, and mild to cooler winter temperatures to trigger final maturation and develop the distinctive aromas of the fungus. Additionally, year-round rainfall, peaking in the winter months will facilitate high levels of production. Within the Site B study area, the impact of differing climatic conditions on truffle yield was seen over the studied harvest seasons. The 2019 season produced significantly less than average yield results, which may have been due to the below average rainfall experienced in the lead up to the harvest season, and the high winter temperatures experienced in the winter months of that year. The 2020 harvest season produced significantly better yield results in which mean monthly rainfall was higher, and more consecutive than the previous year, and the warmer than average summer and cool winter temperatures would have facilitated ideal growing conditions for the 2020 season.

The soil profile created for Site B suggested that soils were too moist to facilitate a high level of truffle production. Highly acidic soils within the study area (those samples that returned pH values less than 6.5) generally produced little to no truffles over the studied harvest years, where more alkaline sample locations (pH values of 7.1 - 7.5) were capable of producing higher yields over the 2019 and 2020 harvest seasons. All 40 soils samples within Site B fell into the loamy sand or sandy loam structure and were therefore suitable for the production of the black truffle. The soil structure (the percentage makeup of sand, silt and clay) across the study area did not vary to the point in which it began to affect truffle yield.

Elements including boron, iron, manganese, magnesium, phosphorous and sodium and zinc had minimum influence over yield in the 2019 and 2020 harvest seasons. Higher truffle yield was associated with soils containing less than 5.1mg/kg of copper. When copper content increased above this amount, truffle yield rapidly declined. Sample locations containing higher nitrogen and sulphur contents consistently produced little to no truffles where sample locations with low nitrogen and sulphur contents consistently produced higher yields of the black truffle over the studied years. Soils with higher concentrations of potassium were correlated with areas of high truffle yield, whereas samples with low concentrations of potassium consistently return little to no truffles in the studied harvest years. These relationships were confirmed through the use of the *Exploratory Regression* tool within ArcGIS. This tool was also used to identify the top three soil properties, as indicated by their R^2 value, influencing harvest season for both the 2019 and 2020 seasons. For the 2019 season, these properties included the soil pH, moisture level and nitrogen content while for the 2020 season, they included the soil nitrogen, copper and sulphur content. These properties were then explored in greater detail using the *Ordinary Least Squares* tool.

This study only provides a basic understanding of the influence of soil properties over truffle yield. The ArcGIS *Ordinary Least Squares* tool found that soil nitrogen and copper content statistically influenced the number of harvest truffles within the Site B study area for the 2019 harvest season. However, in order to determine which soil property / properties have the most influence over yield, and the extent of these relationships, a soil scientist with a greater understanding of soil chemistry is required. Additionally, a soil scientist may assist in identifying whether it is a combination of soil properties, rather than just a single one, that work in symbiosis with each other to produce a higher yield of truffles.

5 CONCLUSIONS & RECOMMENDATIONS

Due to its aromatic quality, rarity and difficulty in cultivation, the Périgord, or as it is more commonly known, the black truffle (*Tuber melanosporum*) is a highly valued commodity worldwide (Bradshaw, 2005). Despite its value to both local and regional economies, knowledge surrounding truffle cultivation within Western Australia is not greatly understood and very limited research has been conducted to explore which factor(s) have the highest influence over truffle yield. Using an industry leading black truffle producing truffière located in the south-west of Western Australia, this research explored spatial variability in truffle yield across the Site B study area. Historical trends showed that within a single truffière, certain trees (known within the industry as "hot" trees) produced more of the black truffle than others, and these trees changed from season to season. Currently, there is little scientific understanding as to what conditions are conducive to a tree becoming "hot" and producing a high truffle yield. Spatial analysis methods, including the *Average Nearest Neighbour Ratio* and *Optimised Hot Spot Analysis*, were utilised to describe and map the spatial distribution of harvested truffles over the 2019, 2020 and 2021 harvest seasons. This spatial distribution was then examined against various soil properties, obtained through a site soil sampling survey, to determine which climatic conditions (rainfall and temperature levels) or soil factors (pH, moisture content, structure and nutrient ratios), had the highest influence over truffle yield. Findings from this research aimed to improve both the utilisation of resources and potentially increase productivity within the truffière, in order to maintain the economic benefits to both the study truffière and the wider south-west region.

5. 1 Research Objectives & Conclusions

To restate, the goal of this research project was to uncover if climatic conditions and soil properties including pH, moisture content, structure and nutrient ratios stimulated truffle production in the Site B study area over the 2019, 2020 and 2021 harvest seasons; or whether the spatial variability in truffle yield exhibited across this site was random and therefore due to individual tree performance. The four key objectives of this research were to:

(a) Describe the macro- and micro- scale spatial variability in truffle yield.
(b) Explore appropriate spatial methods used to describe variability across a study area (e.g. Hot Spot Analysis).
(c) Understand whether the distribution of truffle yield across the chosen study area is random.
(d) Determine whether factors and conditions (such as soil properties) could potentially stimulate truffle production.

The following section will aim to summarise and conclude these research objectives.

5.1.1 Objective 1: Describe the Macro- and Micro- Scale Spatial Variability in Truffle Yield

As discussed in Chapter 2, historically available literature surrounding the *Tuber melanosporum* outlined what conditions have been important to the black truffle within the northern hemisphere. Very few sources have analysed and adapted this historical knowledge to be more applicable for the production of truffles within the southern hemisphere. Hall, Brown and Zambonelli's 2007 text, *Taming the Truffle: The History, Lore and Science of the Ultimate Mushroom*, is one such example that identifies this issue and has attempted this adaptation, and was therefore largely referenced within this research. This text, along within other sources including Bradshaw (2005), Lee (2008) and Mathews & Mitchell (2018), outlines the conditions conducive to black truffle development and growth, as well as suitable climatic and soil environments that would influence the spatial variability in truffle yield.

On a macro (global) scale, for a high yield of the black truffle to occur, a truffière should be located in areas with a Mediterranean climate. Warm summer temperatures are required to initiate truffle formation, while cooler winter temperatures trigger final maturation and develop the distinctive aromas of the fungus (Mathews & Mitchell, 2018). Year-round maintenance of root-zone moisture facilitates high levels of production, and irrigation is required to supplement moisture levels when rainfall does not exceed 1000m annually. Alkaline soils with a pH between 7.2 and 8.3 are preferable, with the highest truffle yield occurring in soils with an optimal pH level of 7.9. Gravelly sandy loam to sandy clay loam surface soils allow for a high truffle yield due to their structure that permits for decent drainage (to reduce root rot), and good aeration. High truffle yield is also dependent upon the soil nutrients, with literature stating that black truffles ideally grows in soils with relatively balanced ratio of nutrients such as "*phosphorus, calcium, magnesium, nitrogen, potassium, sulphur and trace elements of boron, copper, iron, manganese and zinc*" (Hall et al., 2007).

As the black truffle is an ectomycorrhizal (ECM) fungus it grows in symbiosis with another plant. As discussed in Chapter 2, the choice of host plant greatly impacts the variability in truffle yield. Within Site B, varieties of Oak and Hazel were chosen as host plants as they supported this symbiotic relationship. Hazels could additionally be planted in relatively high densities, have a rapid growth rate and a well-developed root system that could easily produce truffles earlier and longer than other plant species. Oaks were chosen as although they have a slower growth rate, and require more space to grow, they provide extensive root systems, increasing the number of truffles per tree, are resilient to cold and require less overall maintenance.

The micro-scale spatial variability in truffle yield can be observed in the Site B study area; a black truffle truffière located in the south-west of Western Australia. The 14.2ha site contains 106 tree rows, with one of the 7,000 inoculated trees planted every 5m along the row, alternating between Oak and Hazel varieties. The study area meet all the required soil and climate conditions, and in consultation with the truffière manager, exhibited a noticeable difference in yield across the site. This variation in yield was further evident in both the 2019, 2020 and 2021 *Harvest Season Optimised Hot Spot Analysis* (Chapter 3 Section *3.3.3.3 Variation in Truffle Yield Site B*) and the 2019 and 2020 *Tree Density Optimised Hot Spot Analysis* (Chapter 4 Section *4.3 Linking High Truffle Yield to Soil*

Properties). High clustering and therefore high truffle yield occurred in areas along the south and south-eastern borders of Site B, while portions of the north-west and north-eastern quadrant contained low clustering and were therefore areas of low truffle yield. These areas of low and high yield were stable across the studied harvest seasons but did slightly vary from season to season. As climatic conditions were consistent across Site B, the micro-scale variation in yield was therefore due other factors, such as soil properties.

5.1.2 Objective 2: Explore Appropriate Spatial Methods used to Describe Variability Across the Study Area and Objective 3: Understand Whether the Distribution of Truffle Yield Across Site B is Random

In order to determine how and why the harvest truffles were spatially distributed across the Site B study area, two key datasets were required. Firstly, the geographical location of each harvested truffle and secondly, a soil profile for the study area. The location (latitude / longitude) dataset was required to record the size and harvest time of each truffle, at a relatively high speed and low cost, with an accuracy of less than 5m, while being able to collect data in remote areas with low Wi-Fi capabilities. This research tested two methods to obtain the required locational data; firstly, a Global Positioning System (GPS) technique, and secondly, a Mobile Data Acquisition (Collector for ArcGIS). A Real Time Kinematic (RTK) GPS approach was unable to provide the required accuracy, due to the amount of tree cover within Site B, which limited the line of site between the base and rover stations, and the GPS satellites. The Mobile Data Acquisition approach was therefore selected for this research as it met all the data requirements.

The soil profile for Site B was obtained through a design-based approach in which the study area was divided up into sample locations randomly selected using the *k-means clustering* method. Due to the restrictions in travel as a result of the Covid-19 pandemic, for this research, 40 samples were chosen as the optimal number of sample locations as it was representative of the study area, while also meeting time and cost restraints. At each of these 40 locations, a 200-500g soil sample was collected at a depth of 0-10cm. These soil samples were then provided to ChemCentre for further analysis who returned the soil pH, moisture level, structure and nutrients for each sample in the study area.

Within this study, two GIS spatial analysis tools were used to determine whether a clear spatial variation in truffle yield existed in the Site B study area, or whether the distribution of yield across Site B was random; The *Average Nearest Neighbour* tool, and the *Optimised Hot Spot Analysis.* The *Average Nearest Neighbour (ANN)* tool from the *Spatial Statistics* toolset calculates the nearest neighbour index to produce the ANN ratio, Z-Score and P-Value for each harvest year (see Chapter 3 Section *3.3.2 Average Nearest Neighbour Ratio* for more information). Within Site B, the ANN reports returned an ANN ratio less than 1 for the 2019, 2020 and 2021 harvest seasons. It was therefore evident that significant level of clustering were present within these datasets, with a high confidence level that this clustering was not due to a random process. This was a clear indication of spatial variability, in which harvested truffles were clustered across the Site B study area.

The *Optimised Hot Spot Analysis* tool was utilised within this study to map the clustering of truffle yield within Site B over the 2019, 2020 and 2021 harvest seasons. This tool was chosen as it used the location of features, rather than a particular attribute, to map statistically significant hot and cold spatial clusters using the Getis-Ord Gi* statistic. With the ideal spatial resolution set at 5m (the distance between the trees within Site B) with the corresponding *Optimised Hot Spot Analysis* maps available in Chapter 3 - Section *3.3.3.3 Variation in Truffle Yield Site B*. For the 2019, 2020 and 2021 harvest seasons, areas of high clustering (i.e. high truffle yield) where shown in red and were consistently located along the south and south-eastern borders of Site B. Areas of low clustering (i.e. little to no truffle yield) were shown in blue and were consistently located in the north-west and north-eastern regions of the study area. Along these hot and cold spots slightly shifted between season to season, they remained relatively stable over the 3 studied harvest seasons. We can therefore say with confidence that a clear spatial variation in truffle yield existed in the Site B study area, and the distribution of yield across Site B is not random.

5.1.3 Objective 4: Understand whether Factors and Conditions (such as Soil Properties) could Potentially Stimulate Truffle Production

This study confirmed that certain climate and soil conditions could influence and potentially stimulate truffle production in the Site B study area. As discussed above, for a truffière to produce a high yield of truffles, it is expected that it would experience year-round rainfall, peaking in the winter months, warm summer temperatures (to initiate the truffle formation) and mild to cooler winter temperatures (to trigger final maturation and develop the distinctive aromas of the fungus). The impact of differing climatic conditions during a harvest year can be seen within the Site B study area, when comparing the 2019 and 2020 harvest seasons. According to the truffière manager, the 2019 season produced significantly less than average yield results. This could be due to the below average rainfall, and high winter temperatures experienced in the lead up to and during the harvest year that would have impeded the ability of the truffles to reach final maturation. 2020 experienced significantly higher yield results than the previous season which could be explained by the higher-than-average mean monthly rainfall (with rainfall falling more consecutive over the year than 2019). Along with the warmer-than-average summer temperatures and the cool winter temperatures experienced that year, ideal climatic growing conditions would have facilitated more truffles to form and mature compared to the previous season.

As discussed in Chapter 2, certain soil conditions are also expected to impact truffle yield. For a high yield to occur, the soil should have:

(a) A pH level between 7.2 and 8.3 (7.9pH is ideal);
(b) A loose structure (soils with high gravel content, e.g. sandy clay loam are ideal); and,
(c) A specific nutrient ratio.

Within this study, a soil profile for Site B was created to by measuring the soil properties at 40 sample locations across the study area. The soil samples taken from these 40 sample locations were provided to a soil testing lab (e.g., ChemCentre), who returned the soil moisture content, pH, particle size (soil structure) and nutrient ratios of each sample. The Site B soil profiles for each of these properties were created by dividing each properties values into 4 groups, using the *Natural Breaks (Jenks)* method, and mapping the results. The soil profiles were then visually compared to the 2019 and 2019 *Tree Density Optimised Hot Spot Analysis* and Table 4.3 *Site B soil sampling locations and their corresponding 2019 and 2020 Tree Density Optimised Hot Spot Analysis values* to identify those soil properties than correlate with areas of high and low truffle yield and eliminate those properties that do not exhibit a clear spatial pattern which links the soil property and truffle yield for each season.

The above analysis suggested that the soils within the Site B study area were generally too moist to facilitate high levels of production over the 2019 and 2020 harvest seasons. Additionally, those sample locations returning highly acidic pH results (values less than 6.5) generally produced little to no truffles, with the higher yields occurring in more alkaline sample locations with pH values between 6.5 and 7.5. All 40 sample locations fell into the ideal loamy sand or sandy loam structure and were therefore suitable for the production of the black truffle. Elements including boron, iron, manganese, magnesium, phosphorous, sodium and zinc visually had no influence over yield in the Site B study area. Areas with low copper, nitrogen and sulphur contents, and high potassium contents also experienced high truffle production over the 2019 and 2020 harvest seasons.

The *Modelling Spatial Relationships* toolset within ArcGIS was used to confirm whether the soil properties identified above (soil moisture, pH, copper, nitrogen, sulphur and potassium contents) influenced truffle yield in the 2019 and 2020 harvest years. The *Exploratory Regression* evaluated the top 3 soil properties that **best** explained the number of harvested truffles within the Site B study area in both the 2019 and 2020 harvest seasons. This tool confirmed that within the

2019 harvest season, nitrogen, copper and sulphur soil contents had a significant negative relationship with truffle yield (i.e. as the content of these elements within the soil increased, the truffle yield decreased), as they returned the highest R^2 values. For the 2020 harvest season, the top 3 influential soil properties included the soil moisture, pH values and nitrogen content. The *Exploratory Regression* tool confirmed that within the 2020 season, moisture and nitrogen content had a significant negative relationship with truffle yield, which pH had a significant positive relationship with truffle yield (i.e. as the soil pH increased, truffle yield also increased).

Although these significant soil properties did not necessarily pass the 0.25 R^2 requirement, the 2019 and 2020 regressions did pass maximum VIF value and minimum spatial autocorrelation p-value tests. The influential soil properties could therefore be explored in greater detail using the *Ordinary Least Squares* tool. This analysis confirmed that:

(a) A strong negative relationship exists between truffle yield and soil nitrogen content (%) in both the 2019 and 2020 harvest seasons;
(b) A strong negative relationship exists between truffle yield and soil copper content (mg/kg) and sulphur content (mg/kg) in the 2019 harvest season:
(c) A strong negative relationship exists between truffle yield and soil moisture (%) in the 2020 harvest season;
(d) A strong positive relationship exists between soil pH and truffle yield in the 2020 season, and:
(e) Nitrogen and sulphur contents were the only statistically significant soil properties to influence truffle yield in the studied years, and this only occurred in the 2019 harvest season.

This study confirmed that certain climate and soil conditions can influence and potentially stimulate truffle production in the Site B study area. Although some soil properties did not pass the *Ordinary Least Squares* check of statistical significance, it does not necessarily mean they don't also play a part in the distribution of truffles over the studied harvest years. In order to determine the extent of which soil property / properties have the most influence over yield, a soil scientist with a greater understanding of soil chemistry is required to further analyse these results. Additionally, a soil scientist may assist in identifying whether it is a combination of soil properties, rather than just a single one, that work in symbiosis with each other to produce a higher yield of truffles in the Site B study area.

5. 2 Limitations, Recommendations & Future Work

This section will explore the limitations involved in this research and the potential management techniques that could be implemented within the study truffière to optimise truffle yield. These techniques will emphasise improving the utilisation of resources and inputs to increase potential harvest from the trees within the study truffière. This thesis will assist in sustaining a high level of production within the truffière, so it can maintain its economic benefits to the surrounding south-west region. Any findings resulting from this research could be further be examined to see (if any) relevance exists to other areas within the agricultural sector of Western Australia, especially in the south-west.

5.2.1 Limitations of Data Capture & Collection

As discussed in Chapter 3, within this study, two methods of obtaining location information were tested; a Global Positioning System (GPS) set-up using a relative positioning technique, and a mobile data acquisition method. The GPS set-up was decided against, as it failed to meet the accuracy requirements for this study, and therefore the locational truffle data was collected using mobile data acquisition. During the harvest seasons, the truffle pickers would use a mobile device to record the geographical, size and harvest time & date of each picked truffle. This locational data allowed us to map the spatial variability of the harvested truffles for the 2019 and 2020 harvest seasons. However, as noted in Chapters 3 & 4, the 2021 harvest season locational data could not be used due to the underutilisation of the mobile recording device by the truffle pickers. This resulted in a severe underreporting of the harvest data, which, if it was utilised in the analysis section of this thesis, could have skewed or influenced the research results.

Due to this underreporting of Site B truffles, the corresponding dataset did not accurately reflect the yield results for 2021 harvest season. However, as discussed in Chapter 4, according to the truffière manager, the 2021 season produced approximately 10,000kgs of truffles, the largest yield of the three years studied, with a higher-than-average number of harvested truffles falling into the "medium" and "large" size categories. This high yield was also backed up by the ideal cool winter temperatures experienced in the June and July of the previous year which would have allowed a higher-than-average yield of truffles to form over the 2021 harvest season. If the mobile data recording device was used to its full potential throughout the harvest season, the dataset could have been used in the analysis. This could have allowed more connections to be made between truffle yield and climate and soil properties and could have potentially strength the findings of this research.

5.2.2 Recommendations

This research has suggested that the soils within the Site B study area were generally too moist to facilitate high levels of production over the 2019 and 2020 harvest seasons. Additionally, within Site B, sample locations returning highly acidic pH (values less than 6.5) generally produced little to no truffles, and higher yields occurred in more alkaline sample locations with pH values between 6.5 and 7.5. Areas with low copper, nitrogen and sulphur contents, and high potassium contents also experienced high truffle production over the 2019 and 2020 harvest seasons. These relationships were confirmed through the use of the *Ordinary Least Squares* tool. Recommendations for the study truffière will aim to improve both the utilisation of resources and potentially increase productivity within the truffière. These recommendations include;

(a) Reducing the level of moisture within Site B. Suggested soil management strategies include looking at irrigation techniques and improving drainage within the study site.

(b) Increasing the pH of the soils within Site B, through techniques such as liming (adding large quantities of calcium carbonate to the soil).

(c) Adjusting the nutrient ratios to reduce amount of copper, nitrogen and sulphur within the soil. Contracting a soil scientist to complete an in-depth soil analysis would allow for more specific recommendations to be made.

These recommendations should allow the truffière to increase truffle productivity and maintain economic benefits to both the study truffière and the wider south-west region.

5.2.3 Future Work

This thesis confirmed the hypothesis that certain climate and soil conditions can influence and stimulate truffle production in the study truffière. Within Site B, areas of low nitrogen, copper, sulphur and moisture contents produced a high yield of truffles over the studied 2019 and 2020 harvest seasons. Additionally, areas of high pH also stimulated yield over these time periods. Although other measured soil properties did not pass this check of statistical significance, it does not necessarily mean they do not play a part in the distribution of truffles over the studied harvest years. In order to determine the extent of which soil property, or properties have the most influence over yield, a soil scientist with a greater understanding of soil chemistry is required to further analyse the analysis results. Additionally, a soil scientist may assist in identifying whether it is a combination of soil properties, rather than just a single one, that work in symbiosis with each other to produce a higher yield of truffles in the Site B study area.

Both the truffle harvest data and soil profile should be regularly recorded and mapped to better identify which soil properties are stimulating truffle yield within Site B. For each season, every harvested truffle's location, size and pick date and time should be recorded. The truffière should focus on efforts to increase the utilisation of the mobile data recording device and ensuring that the truffle pickers understand the importance of the harvest data. A soil profile, like the one completed in this research should be undertaken every three to five years to monitor and map the changes in soil properties within the study area. These soil profiles will assist the truffière in making informed decisions surrounding the management of soil properties, in order to stimulate truffle yield within the study truffière. Any findings resulting from this research could additionally be examined further to see (if any) relevance exists to other areas within the agricultural sector of Western Australia, especially in the south-west.

REFERENCES

AgriFutures Australia. (2017). Truffles - Overview - Facts and Figures. Retrieved from https://www.agrifutures.com.au/farm-diversity/truffles/

Amaranthus, M. (2007). Independent Truffle Expert's Report in Production Disclosure Statement for the Oak Valley Truffle Project 2007. *ARSN:124 296 028*, 26 - 34.

Bad Elf. (2020). Bad Elf GNSS Surveyor Specifications. Retrieved from https://bad-elf.com/pages/be-gps-3300-detail

Bakker, W., Feringa, W., Gieske, A. S. M., Gorte, B. G. H., Grabmaier, K. A., Hecker, C. A., . . . Woldai, T. (2009). *Principles of remote sensing: an introductory textbook (4th Ed.)* (K. Tempfli, N. Kerle, G. C. Huurneman, & L. L. F. Janssen Eds. 4 ed.). Enschede, The Netherlands: The International Institute for Geo-Information Science and Earth Observation.

Bakuła, M., Oszczak, S., & Pelc-Mieczkowska, R. (2009). Performance of RTK Positioning in Forest Conditions: Case Study. *Journal of Surveying Engineering, 135*(3), 125-130. doi:doi:10.1061/(ASCE)0733-9453(2009)135:3(125)

Ball, J. (1999). Understanding and Correcting Soil Acidity *Noble Research Institute Ag News and Views*.

Bradshaw, B. P. (2005). *Physiological aspects of Corylus avellana associated with the French black truffle fungus Tuber melanosporum and the consequences for commercial production of black truffles in Western Australia.* (Doctor of Philosophy). Murdoch University, Perth, Western Australia.

Brus, D. (2010). Design-based and model-based sampling strategies for soil monitoring. *19th World Congress of Soil Science, Soil Solutions for a Changing World*, 32-34.

Brus, D. (2020). Statistical approaches for spatial sample survey: Persistent misconceptions and new developments. *European Journal of Soil Science, n/a*(n/a). doi:10.1111/ejss.12988

Brus, D., & de Gruijter, J. (1997). Random sampling or geostatistical modelling? Choosing between design-based and model-based sampling strategies for soil (with discussion). *Geoderma, 80*(1), 1-44. doi:https://doi.org/10.1016/S0016-7061(97)00072-4

Castrignanò, A., Goovaerts, P., Lulli, L., & Bragato, G. (2000). A geostatistical approach to estimate probability of occurrence of Tuber melanosporum in relation to some soil properties. *Geoderma, 98*(3), 95-113. doi:https://doi.org/10.1016/S0016-7061(00)00054-9

Churchward, H. M. (1992). *Soils and landforms of the Manjimup area, Western Australia / by H.M. Churchward ; editors L. J. Snell and B. Hillman*. South Perth, W.A: Dept. of Agriculture, Western Australia.

Craze, B. (1990). Soil Survey Standard Test Method: Soil Moisture Content. *Department of Sustainable Resources* 1-5.

de Gruijter, J., Brus, D., Bierkens, M., & Knotters, M. (2006). *Sampling for Natural Resource Monitoring*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Department of Agriculture and Food (Cartographer). (2012). Characteristic Soils of South Western Australia

DSNR. (2002). Soil Survey Standard Test Method: Particle Size Analysis. *Department of Sustainable Resources*, 1-8.

El-Rabbany, A. (2006). *Introduction to GPS: The Global Positioning System (Second Edition)*. Norwood, MA: Artech House.

Environmental Systems Research Institute (ESRI). (2020). Collector for ArcGIS. Retrieved from https://esriaustralia.com.au/collector-for-arcgis

Environmental Systems Research Institute (ESRI). (2021). ArcGIS Pro Help 2.8 How Multivariate Clustering Works. Retrieved from https://pro.arcgis.com/en/pro-app/latest/tool-reference/spatial-statistics/how-multivariate-clustering-works.htm

Environmental Systems Research Institute (ESRI). (2022). ArcGIS Pro Help 2.9 Regression Analysis Basics. Retrieved from https://pro.arcgis.com/en/pro-app/latest/tool-reference/spatial-statistics/regression-analysis-basics.htm

Fondriest Environmental Products. (2020). Eos Arrow Lite GPS Receiver. Retrieved from https://www.fondriest.com/eos-arrow-lite-gps-receiver.htm

Garmin. (2020). Sensors: GLO 2 Device Specs. Retrieved from https://buy.garmin.com/en-AU/AU/p/645104#specs

Geneq Inc. (2018). iSXblueII + GPS Fact Sheet. Retrieved from https://sxbluegps.com/wp-content/uploads/2019/02/iSXBlue-II-GPS-EN-web-1.pdf

Grekousis, G. (2020). Spatial Analysis Methods and Practice. In G. Grekousis (Ed.), *Spatial Analysis Methods and Practice: Describe Explore Explain through GIS*. Cambridge: Cambridge University Press.

Hall, I., Brown, G., & Zambonelli, A. (2007). *Taming the Truffle: The History, Lore and Science of the Ultimate Mushroom*. Portland, Or: Timber Press.

Hall, I., & Haslam, W. (2012). Truffle Cultivation in the Southern Hemisphere. In A. Zambonelli & G. Bonito (Eds.), *Edible Ectomycorrhizal Mushrooms: Current Knowledge and Future Prospects* (Vol. 34, pp. 191-208): Springer.

John Hopkins Sheridan Libraries. (2017). Collector for ArcGIS. *Library GIS and Map Guides*. Retrieved from https://guides.library.jhu.edu/gis/collector

Kuhn, M. (2018). Chapter 4: GPS Positioning Principles. *SPAT2006: Applications of Global Positioning Systems Course Notes*.

Kuhn, M. (2018a). Chapter 8: A Review of Accuracy and Precision. *SPAT2006: Applications of Global Positioning Systems Course Notes*.

Kuhn, M. (2018b). Part V - Lecture: Surveying with GPS (18th April 2018). *SPAT2006: Applications of Global Positioning Systems Course Notes*.

Kuhn, M. (2018c). Real-Time Kinematic Positioning Using GNSS - A Basic Introduction. *Practical Notes - SPAT2006 Application of Global Positioning Systems - Semester One 2018*.

Lake, B., & New South Wales Agriculture. (2000). Understanding soil pH. *New South Wales Acid Soil Action Program - Leaflet No. 2*. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/167187/soil-ph.pdf

Lee, B. (2008). *Taking Stock of the Australian Truffle Industry*. Kingston, ACT: Canprint

Linde, C. C., & Selmes, H. (2012). Genetic Diversity and Mating Type Distribution of *Tuber Melanosporum* and Their Significance to Truffle Cultivation in Artifically Planted Trurrieres in Australia. *Applied and Environmental Microbiology, 78*(18), 6534 - 6539.

Mathews, A., & Mitchell, A. (2018). Cultivation of black truffles in Western Australia. *Department of Primary Industries and Regional Development's Agriculture and Food*. Retrieved from https://www.agric.wa.gov.au/new-horticulture-crops/cultivation-black-truffles-western-australia

McArthur, W. M. (2004). *Reference soils of south-western Australia / [by] W.M. McArthur ; edited by D.A.W. Johnston, and L.J. Snell*. Perth, W.A: Dept. of Agriculture Western Australia on behalf of the Australian Society of Soil Science Inc.

McAuliffe, E. (2019). How Australia is shaking up the truffle market. *BBC News*.

Mehlich, A. (1984). Mehlich 3 soil text extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis, 15*(12), 1409-1416.

Pánek, J., & Glass, M. (2018). Gaining a mobile sense of place with collector for ArcGIS. *Journal of Geography in Higher Education, 42*(4), 603-616.

Papritz, A., & Webster, R. (1995a). Estimating temporal change in soil monitoring: I. Statistical theory. *European Journal of Soil Science, 46*(1), 1-12. doi:10.1111/j.1365-2389.1995.tb01808.x

Papritz, A., & Webster, R. (1995b). Estimating temporal change in soil monitoring: II. Sampling from simulated fields. *European Journal of Soil Science, 46*(1), 13-27. doi:10.1111/j.1365-2389.1995.tb01809.x

Pimpler, E. (2017). *Spatial Analytics with ArcGIS: Use the spatial statistics tools provided by ArcGIS and build your own to perform complex geographic analysis*. Birmingham, United Kingdom: Packt Publishing.

Pundt, H., & Brinkkötter-Runde, K. (1998). Visualisation of spatial data for field based GIS. *Computers & Geosciences, 26*, 51-56

Queensland Government (Environment Land & Water). (2013). Soil Management - Soil Properties: Soil pH Retrieved from https://www.qld.gov.au/environment/land/management/soil/soil-properties/ph-levels

Renowden, G. (2005). *The Truffle Book, Chapter 4 - The World of Truffles: France*. Amberley, New Zealand: Limestone Hills Publishing.

Reyna, S., & Garcia-Barreda, S. (2014). Black truffle cultivation: a global reality. *Forest Systems, 23*(2), 317 - 328.

Rural Industries Research and Development Corporation (RIRDC). (2014). Emerging animal and plant industries: Their value to Australia. *RIRDC Pulication No. 14/069*.

Shaw, P., Lankey, K., & Jourdan, A. (1996). *Factors affecting yield of Tuber melanosporum in a Quercus ilex plantation in southern France* (Vol. 100).

Study Truffiere. (2018). Welcome to [Name of Study Truffiere].

Trimble. (2019). Trimble R1 GNSS Receiver Datasheet. Retrieved from https://geospatial.trimble.com/sites/default/files/2019-10/Datasheet%20-%20Trimble%20R1%20GNSS%20receiver%20-%20English%20%28US%29%20-%20Screen.pdf

Trufficulture Pty Ltd. (n.d.). Trufficulture Nuseries - Growing Truffles. Retrieved from https://trufficulture.com.au/growing-truffles/#establishing

Viscarra Rossel, R., Brus, D., Lobsey, C., Shi, Z., & McLachlan, G. (2016). Baseline estimates of soil organic carbon by proximal sensing: Comparing design-based, model-assisted and model-based inference. *Geoderma, 265*, 152-163. doi:https://doi.org/10.1016/j.geoderma.2015.11.016

Watershed Premium Wines Ltd. (2007). Oak Valley Project 2007. *Product Disclosure Statement / Prospectus, ARSN 124 296 028*.

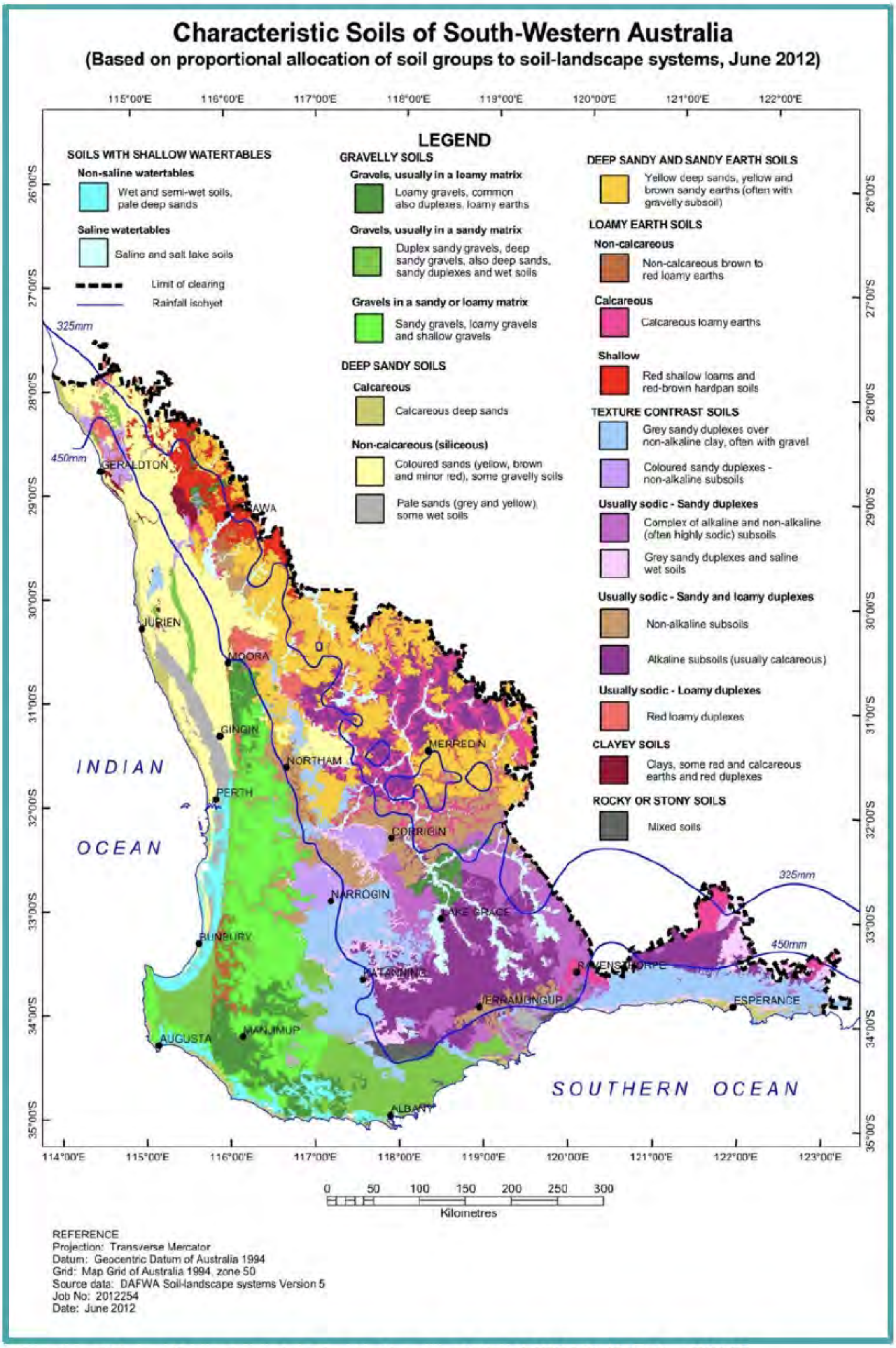
Webster, R., & Lark, R. M. (2012). *Field sampling for environmental science and management*.

Zambonelli, A., Iotti, M., & Hall, I. (2015). Current status of truffle cultivation: recent results and future perspectives. *Micologia Italiana, 44*, 31-40. doi:https://doi.org/10.6092/issn.2465-311X/5593

Zambonelli, A., Piattoni, F., Iotti, M., & Hall, I. (2010). What makes a good truffle infected tree? *Acta Botanica Yunnanica, 16*, 86-89.

APPENDICIES

APPENDIX A: Characteristic Soils of the South-Western Australia



Retrieved from Department of Agriculture and Food (2012) [01 May 2019]

APPENDIX B: Examples of GPS receivers used to increase the accuracy of the *Collector for ArcGIS* application

GPS Receiver Name	Accuracy Level	Battery Life	Bluetooth Connectivity	Compatibility	Cost (May 2020)
Geneq iSXblue II + GPS 	~60cm accuracy with DGPS, 10mm with additional RTK option	8hrs	Yes	iOS, Android & Windows	$1,895USD (not including RTK)
Trimble R1 Integrated GNSS System 	~50cm with ViewPoint RTX service	All Day	Yes	iOS, Android & Windows	~$2,500USD + $400AUD for 1yr subscription to ViewPoint RTX
Bad Elf GNSS Surveyor 	1m accuracy (developed with ArcGIS Collector in Mind)	All Day	Yes	iOS, Android & Windows	$599.99 USD
Eos Arrow Lite Submeter GPS 	~60cm accuracy with free SBAS	15hr	Yes	iOS, Android & Windows	$1,995USD
Garmin GLO™ 2 	3m accuracy	13hr	Yes	iOS, Android	$149AUD

Data and Images available from: (Bad Elf, 2020; Fondriest Environmental Products, 2020; Garmin, 2020; Geneq Inc, 2018; Trimble, 2019)

APPENDIX C: Supporting images for the preparation of data using the *Collector for ArcGIS* application

Image 1: Creating an automatically added **date** field to the *harvestedtruffles* feature class

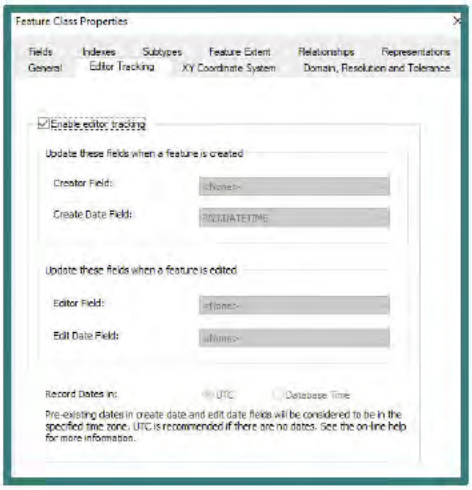


Image 2: Creating and adding a domain to the **Size** field.

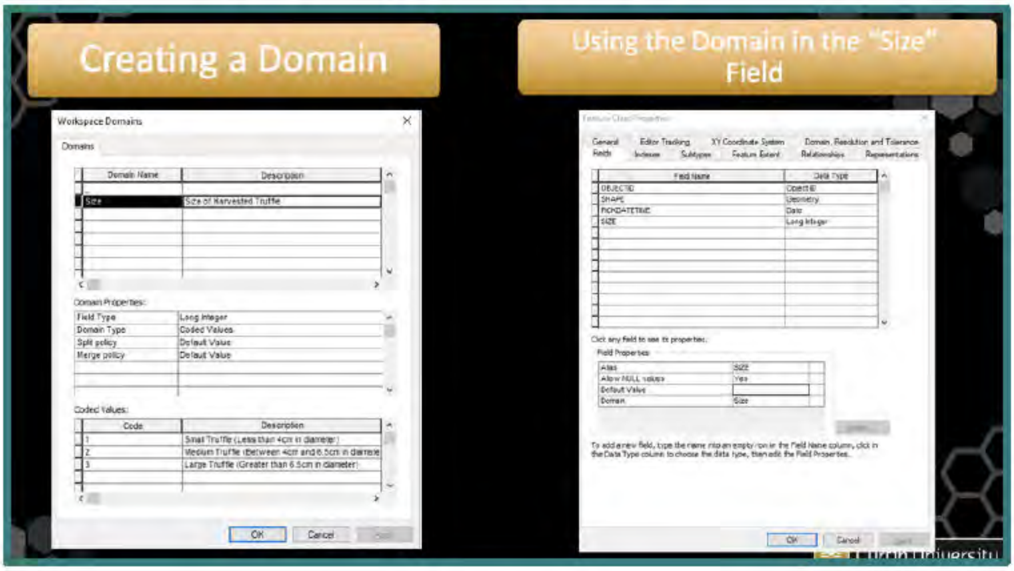
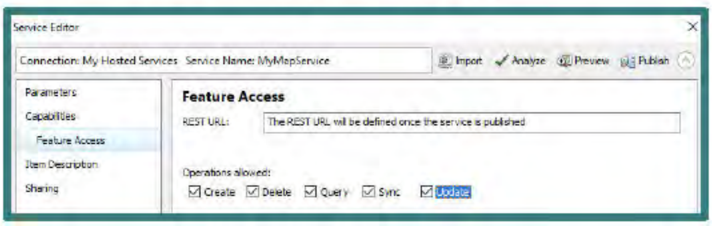


Image 3: Saving and sharing map as service, ensuring all operations are allowed for use in the *Collector for ArcGIS* app.



APPENDIX D: Supporting images for the collection of location data in the field using the *Collector for ArcGIS* application

Image 1: Example point obtained using the *Collector for ArcGIS* app, and the corresponding data

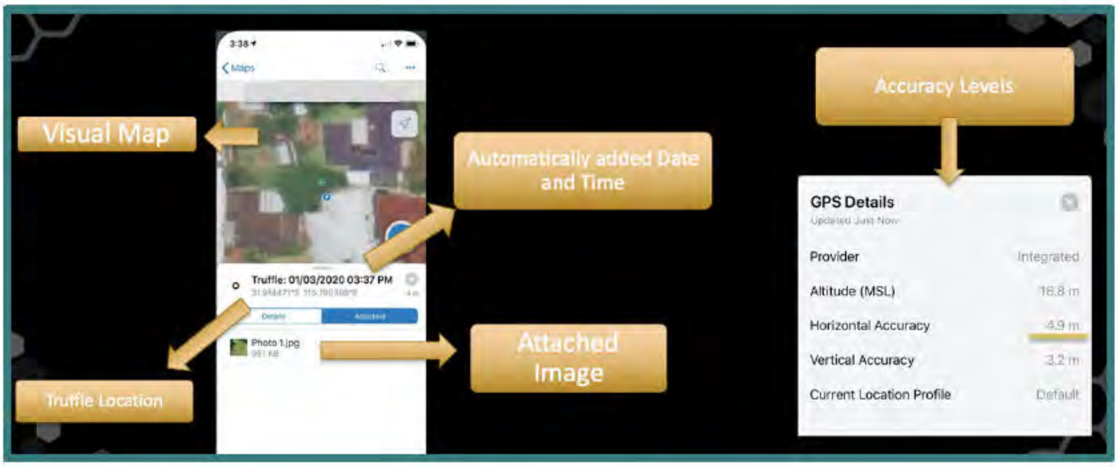
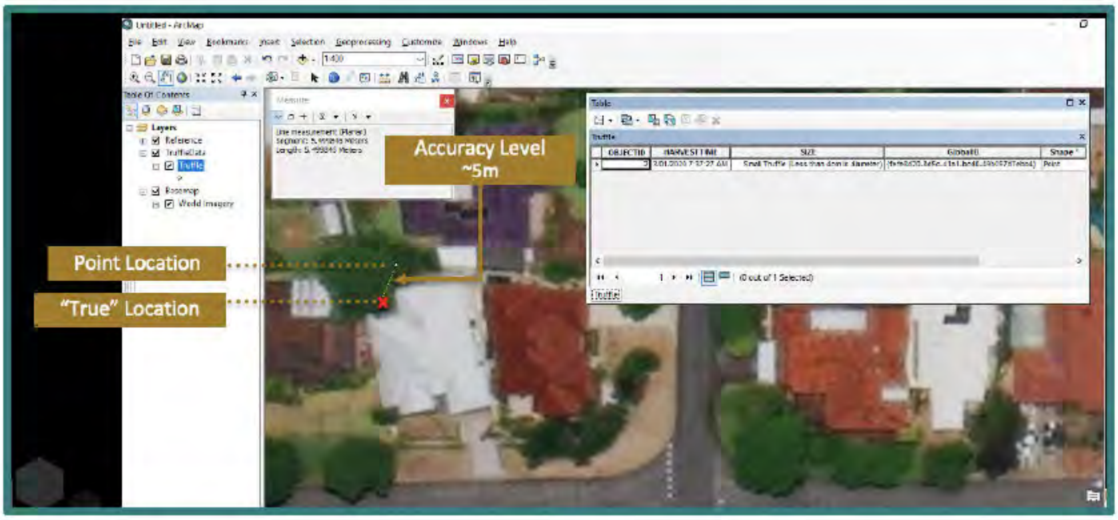


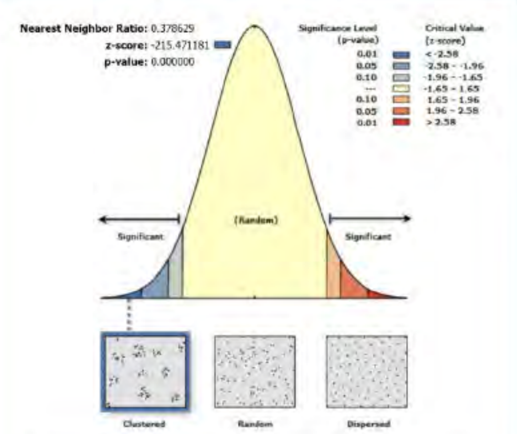
Image 2: *Harvestedtruffles* feature layer reloaded into *ArcGIS* for further spatial analysis



APPENDIX E: Average Nearest Neighbour summary reports

Report 1: 2019 Harvest Season

Average Nearest Neighbor Summary



Given the z-score of -215.471181127, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

Average Nearest Neighbor Summary

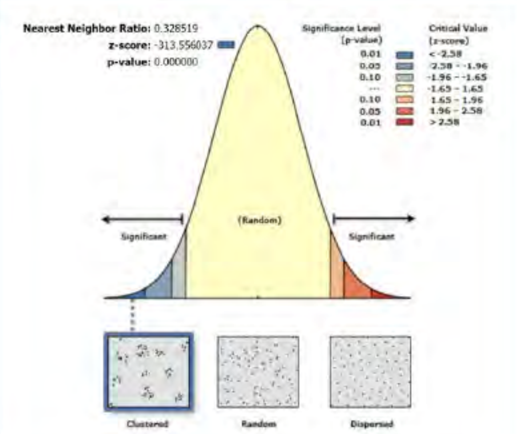
Observed Mean Distance:	0.5121 Meters
Expected Mean Distance:	1.3525 Meters
Nearest Neighbor Ratio:	0.378629
z-score:	-215.471181
p-value:	0.000000

Dataset Information

Input Feature Class:	SiteB_Truffle2019
Distance Method:	EUCLIDEAN
Study Area:	240399.325743
Selection Set:	False

Report 2: 2020 Harvest Season

Average Nearest Neighbor Summary



Given the z-score of -313.556036549, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

Average Nearest Neighbor Summary

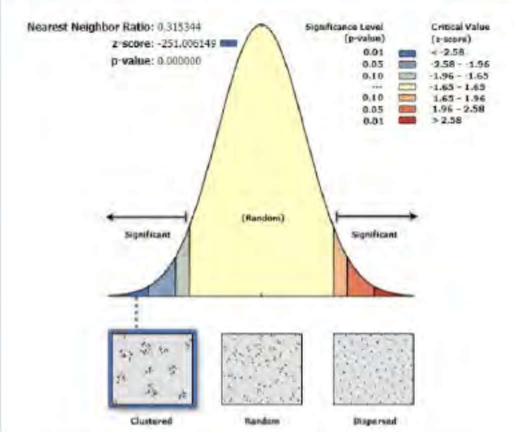
Observed Mean Distance:	0.3273 Meters
Expected Mean Distance:	0.9964 Meters
Nearest Neighbor Ratio:	0.328519
z-score:	-313.556037
p-value:	0.000000

Dataset Information

Input Feature Class:	SiteB_Truffle2020
Distance Method:	EUCLIDEAN
Study Area:	236597.372861
Selection Set:	False

Report 3: 2021 Harvest Season

Average Nearest Neighbor Summary



Given the z-score of -251.006149491, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

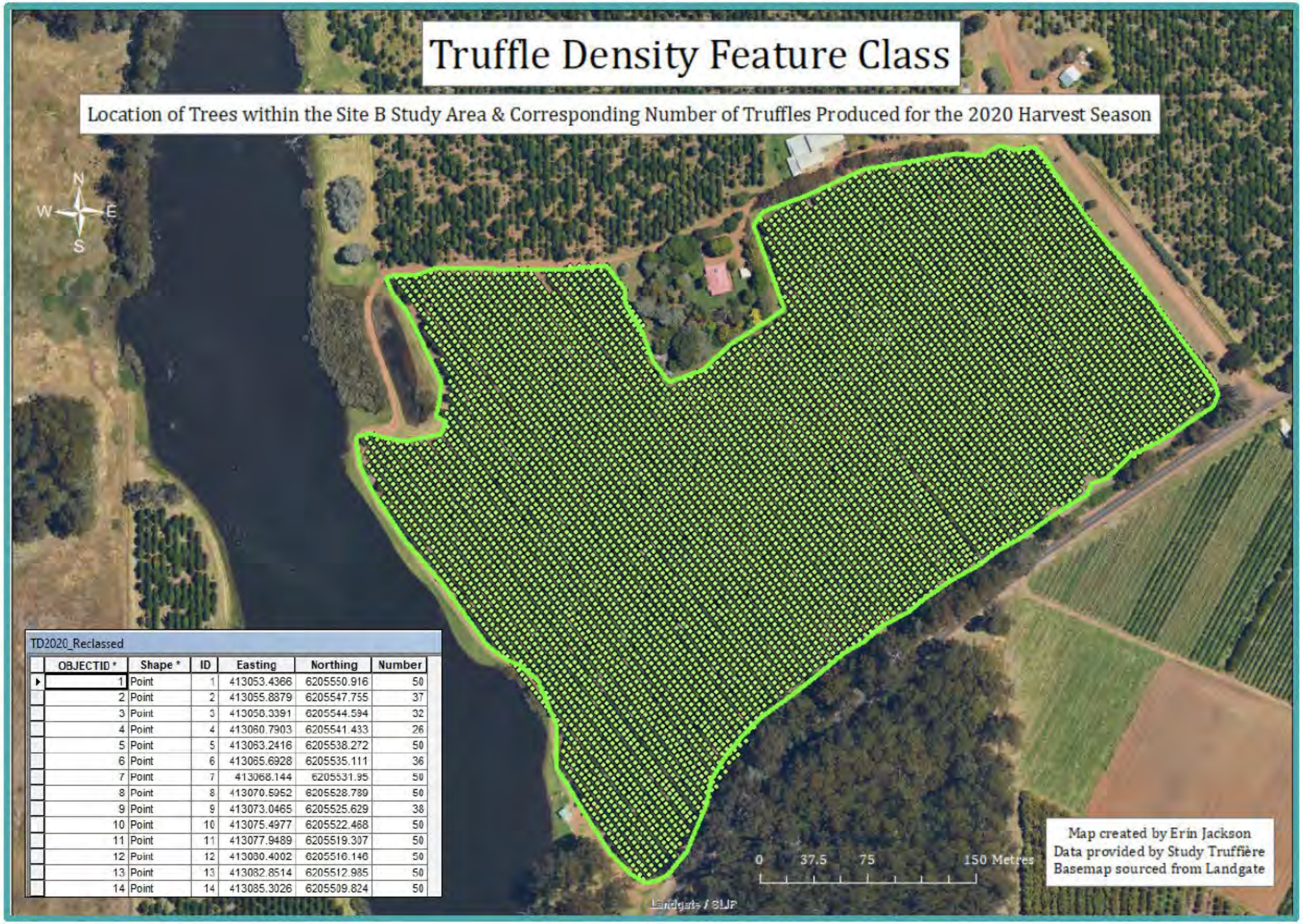
Average Nearest Neighbor Summary

Observed Mean Distance:	0.4041 Meters
Expected Mean Distance:	1.2813 Meters
Nearest Neighbor Ratio:	0.315344
z-score:	-251.006149
p-value:	0.000000

Dataset Information

Input Feature Class:	SiteB_Truffles2021
Distance Method:	EUCLIDEAN
Study Area:	241181.905880
Selection Set:	False

APPENDIX F: *Truffle density* feature class containing the location (easting & northing) of every tree within the Site B study area, and its corresponding number of truffles produced during the 2020 harvest season

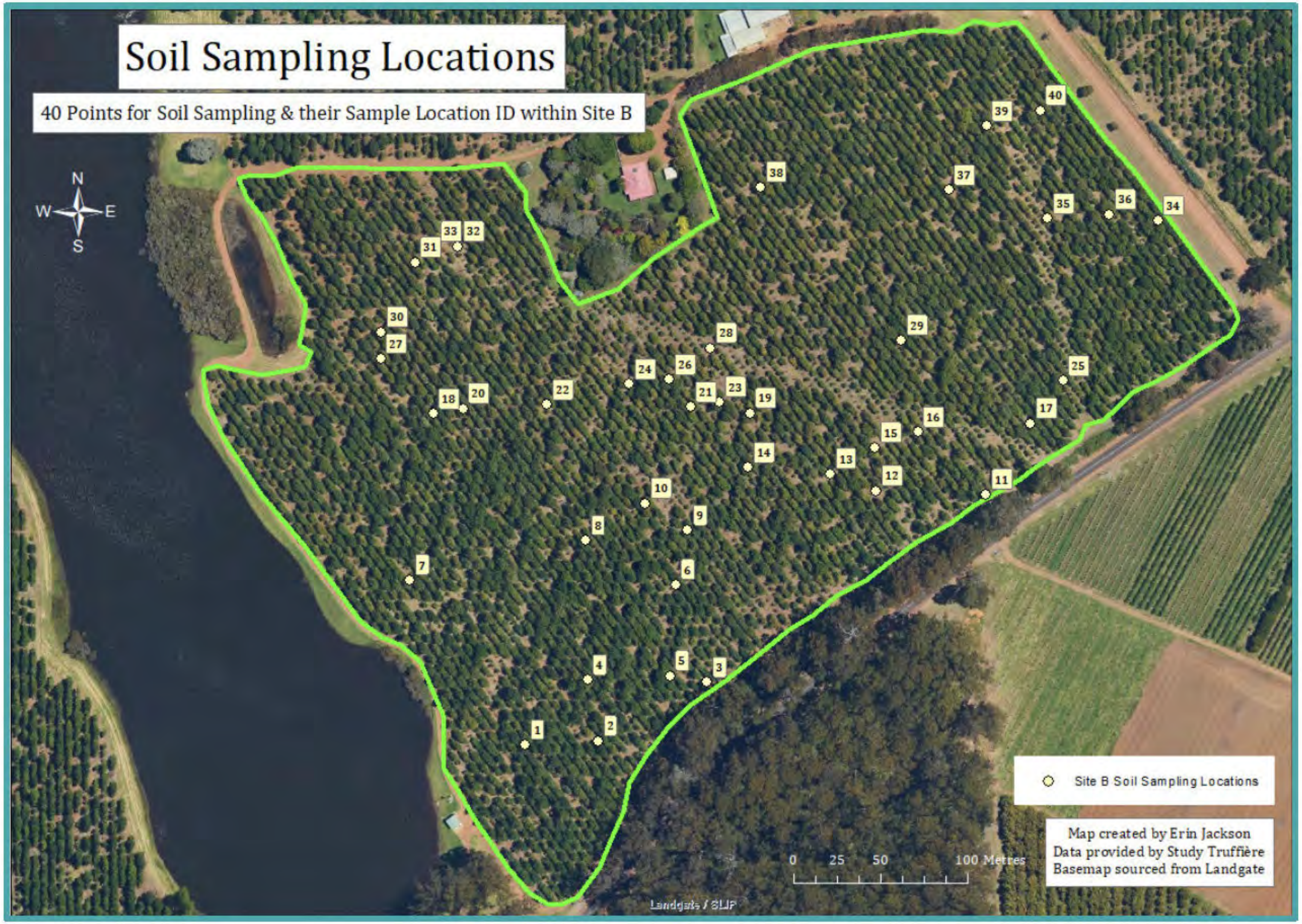


OBJECTID *	Shape *	ID	Easting	Northing	Number
1	Point	1	413053.4366	6205550.916	50
2	Point	2	413055.8879	6205547.755	37
3	Point	3	413058.3391	6205544.594	32
4	Point	4	413060.7903	6205541.433	26
5	Point	5	413063.2416	6205538.272	50
6	Point	6	413065.6928	6205535.111	36
7	Point	7	413068.144	6205531.95	50
8	Point	8	413070.5952	6205528.789	50
9	Point	9	413073.0465	6205525.629	38
10	Point	10	413075.4977	6205522.468	50
11	Point	11	413077.9489	6205519.307	50
12	Point	12	413080.4002	6205516.146	50
13	Point	13	413082.8514	6205512.985	50
14	Point	14	413085.3026	6205509.824	50

APPENDIX G: Soil sampling points and their corresponding sample location number, tree ID, easting and northing

Sample Location Number	Tree ID	Easting	Northing
1	6880	412769.115	6205133.613
2	6316	412811.4563	6205135.899
3	5081	412874.518	6205169.87
4	6074	412805.155	6205171.019
5	5364	412852.8067	6205173.523
6	4697	412856.2174	6205226.317
7	6765	412702.3961	6205228.935
8	5149	412804.1362	6205251.958
9	4250	412863.2268	6205257.675
10	4418	412838.5577	6205273.478
11	2370	413035.9101	6205278.553
12	2936	412972.0416	6205280.701
13	3093	412945.566	6205290.465
14	3469	412898.1729	6205294.256
15	2769	412971.662	6205305.706
16	2489	412996.3012	6205314.602
17	1741	413061.6531	6205319.811
18	5611	412715.8428	6205325.29
19	3244	412899.1791	6205325.722
20	5315	412732.985	6205328.437
21	3512	412864.7585	6205329.242
22	4575	412781.6176	6205330.529
23	3350	412881.5648	6205332.142
24	3808	412829.4675	6205342.392
25	1333	413080.4759	6205344.371
26	3507	412852.5049	6205345.074
27	5691	412685.3486	6205357.134
28	3178	412876.2721	6205363.477
29	2134	412986.7385	6205367.819
30	5496	412685.7714	6205372.686
31	4728	412705.2922	6205412.975
32	4285	412729.9439	6205422.213
33	4458	412717.1395	6205422.254
34	89	413135.2547	6205437.214
35	663	413071.3092	6205438.325
36	308	413107.1818	6205440.696
37	1100	413014.2003	6205454.89
38	2244	412905.2112	6205456.514
39	585	413036.0162	6205491.956
40	233	413067.1301	6205501.064

APPENDIX H: Map of soil sampling locations within the Site B study area



APPENDIX I: Complete soil analysis report provided by ChemCentre for the Site B study area

ChemCentre ID	Client ID	Sample Date	H2O_105C	H2O_40C	pH	Sand.	Silt.	Clay.	N	Al	B	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn	As	Pb	Se
Method Code			Moisture	Moisture	(CaCl2)	fraction	fraction	fraction	(combs)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)	(M3)
Limit of Reporting			0.1	0.1	0.1	0.5	0.5	0.5	0.005	1	0.1	10	0.01	0.01	0.1	1	1	10	0.05	0.01	1	0.1	1	1	0.1	0.1	0.1	0.1
Units			%ar	%ar		%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
21S1345/001	1	28/9/21	29.8	27	7	76	16	8	0.45	>550	1.2	5100	0.15	0.02	2	43	200	370	6.7	<0.01	62	0.1	12	14	1.1	<0.1	0.3	<0.1
21S1345/002	2	28/9/21	31.1	28.5	7.1	76.5	16	7.5	0.46	>550	1.3	>5500	0.18	0.02	1.8	56	150	390	5.8	0.01	52	0.1	21	14	1.5	<0.1	0.3	<0.1
21S1345/003	3	28/9/21	26	24.1	5.2	80.5	13	6.5	0.36	>550	0.5	1000	0.11	0.02	1	68	130	150	5.5	0.02	40	0.1	10	14	0.4	<0.1	0.2	<0.1
21S1345/004	4	28/9/21	29.9	27.4	7.3	76	17	7	0.51	>550	1.5	>5500	0.18	0.03	3.7	43	210	530	7	<0.01	65	0.1	12	16	1.3	<0.1	0.6	<0.1
21S1345/005	5	28/9/21	28.7	26.4	6.8	78.5	15	6.5	0.45	>550	1.1	>5500	0.18	0.02	1.6	46	210	450	6	<0.01	64	0.1	15	19	1.2	<0.1	0.4	<0.1
21S1345/006	6	28/9/21	28.9	27.1	6.8	77	16	7	0.46	>550	1.1	4700	0.16	0.02	1.8	39	140	390	6.2	<0.01	52	<0.1	13	11	1	<0.1	0.4	<0.1
21S1345/007	7	28/9/21	29	27.1	6	65	18	17	0.48	>550	0.7	2400	0.14	0.04	0.9	78	87	330	13	<0.01	46	<0.1	13	13	0.5	<0.1	0.4	<0.1
21S1345/008	8	28/9/21	27.2	25.1	6.9	77.5	14	8.5	0.41	>550	1.1	4600	0.15	0.03	1.5	43	160	330	7	<0.01	49	<0.1	23	11	1	0.1	0.3	<0.1
21S1345/009	9	28/9/21	27.2	23.9	7.1	76	17	7	0.48	>550	1.1	>5500	0.17	0.02	1.9	42	130	520	4.2	<0.01	63	0.1	11	12	0.8	<0.1	0.2	<0.1
21S1345/010	10	28/9/21	29.3	26.1	6.9	77	16	7	0.57	>550	1.4	>5500	0.21	0.02	4	42	160	450	6.5	<0.01	54	0.1	24	11	1.6	<0.1	0.3	<0.1
21S1345/011	11	28/9/21	32.5	29.9	7.2	76	17	7	0.55	>550	1.6	>5500	0.19	0.02	0.8	34	91	440	7.8	<0.01	61	0.1	9	11	1.1	<0.1	0.2	<0.1
21S1345/012	12	28/9/21	24.6	22.2	7.2	80	15	5	0.44	>550	1.3	>5500	0.16	0.01	1.2	32	140	410	4.9	<0.01	52	0.1	10	10	1	<0.1	0.2	<0.1
21S1345/013	13	28/9/21	26.3	22.4	7.5	75	18	7	0.64	>550	2.3	>5500	0.22	0.02	1.4	36	150	670	9.6	<0.01	75	0.2	17	20	1.9	0.1	0.2	<0.1
21S1345/014	14	28/9/21	26.8	23.7	7.3	73	19	8	0.58	>550	1.6	>5500	0.19	0.03	5.1	42	180	520	7.7	<0.01	79	0.1	15	15	1.2	<0.1	0.2	<0.1
21S1345/015	15	28/9/21	25.9	23.1	7.2	78	15	7	0.46	>550	1.2	>5500	0.16	0.02	2.5	35	74	370	5.6	<0.01	46	0.2	11	12	1	0.1	0.3	<0.1
21S1345/016	16	28/9/21	26.5	23.7	7.2	77	15	8	0.4	>550	1	>5500	0.15	0.02	1.2	38	82	280	4.7	<0.01	45	0.2	9	10	0.7	<0.1	0.4	<0.1
21S1345/017	17	28/9/21	30.6	27.3	7.1	73	19	8	0.45	>550	1.3	>5500	0.15	0.02	0.8	34	160	420	5.6	<0.01	67	0.1	7	14	0.8	<0.1	0.2	<0.1
21S1345/018	18	28/9/21	23	20.1	6.7	69	21	10	0.6	>550	0.9	>5500	0.22	0.02	1.4	54	100	470	7.6	<0.01	59	0.2	34	14	1.6	0.1	0.4	<0.1

21S1345/019	19	28/9/21	25.2	22.6	7.3	74.5	18.5	7	0.57	>550	1.4	>5500	0.21	0.02	1.3	39	130	520	8.5	<0.01	67	0.2	20	17	1.7	<0.1	0.3	<0.1
21S1345/020	20	28/9/21	20	16.6	6.6	76	17.5	6.5	0.67	>550	1	>5500	0.22	0.03	2.2	72	170	520	9.6	<0.01	63	0.2	56	18	2.4	<0.1	0.4	0.1
21S1345/021	21	28/9/21	31.1	28.7	7.2	60	21	19	0.49	>550	1.3	>5500	0.19	0.04	1.3	42	110	510	10	<0.01	60	0.1	25	17	1.4	<0.1	0.3	<0.1
21S1345/022	22	28/9/21	20.9	18.2	6.9	70	20	10	0.52	>550	0.8	>5500	0.2	0.02	1.8	58	59	420	7.3	<0.01	42	0.2	51	13	2.1	<0.1	0.4	<0.1
21S1345/023	23	28/9/21	25.9	22.9	7.3	75	17	8	0.44	>550	1.2	>5500	0.19	0.03	1.4	38	94	410	5.8	<0.01	51	0.2	18	15	1.3	<0.1	0.3	<0.1
21S1345/024	24	28/9/21	21.4	18.2	7.2	77	16	7	0.59	>550	1.1	>5500	0.21	0.02	2	48	78	540	8.3	<0.01	49	0.2	47	20	2.1	<0.1	0.4	<0.1
21S1345/025	25	28/9/21	32	29.4	7.1	76	17	7	0.44	>550	1.3	>5500	0.16	0.02	0.7	31	98	350	5.5	<0.01	61	0.1	10	15	1.1	<0.1	0.2	<0.1
21S1345/026	26	28/9/21	26.6	24.6	7.3	74	17	9	0.59	>550	1.5	>5500	0.2	0.02	1.6	45	130	510	6.9	<0.01	57	0.2	26	19	1.7	0.1	0.3	<0.1
21S1345/027	27	28/9/21	29.9	27	6.2	71.5	20	8.5	0.82	>550	1	4800	0.23	0.02	2.4	64	170	490	7.8	<0.01	73	0.2	16	13	2.1	<0.1	0.2	<0.1
21S1345/028	28	28/9/21	27.1	24.8	7.5	67	20	13	0.53	>550	1.5	>5500	0.2	0.02	1.1	44	87	520	8.9	<0.01	56	0.1	21	18	1.9	<0.1	0.3	<0.1
21S1345/029	29	28/9/21	28.4	25.9	7	79	14	7	0.45	>550	1.2	>5500	0.16	0.02	0.7	33	110	420	4	<0.01	55	0.2	12	17	0.9	<0.1	0.2	<0.1
21S1345/030	30	28/9/21	30.2	26.5	6.2	69	23	8	0.81	>550	0.7	4700	0.2	0.03	3	63	130	420	6.1	<0.01	67	0.2	13	13	1.4	<0.1	0.2	<0.1
21S1345/031	31	28/9/21	32.4	29.3	6.6	70	22	8	0.81	>550	1.2	>5500	0.25	0.03	3.3	56	180	580	5.9	<0.01	76	0.1	16	14	1.7	<0.1	0.1	<0.1
21S1345/032	32	28/9/21	30.2	28	6.2	75	18	7	0.82	>550	0.7	5300	0.26	0.03	6.3	71	67	430	6.6	<0.01	57	0.2	34	13	2.8	<0.1	0.2	<0.1
21S1345/033	33	28/9/21	32.6	30.4	6.9	73	19	8	0.88	>550	1.1	>5500	0.25	0.03	3.2	62	150	480	7.4	<0.01	56	0.2	22	18	2.1	<0.1	0.2	<0.1
21S1345/034	34	28/9/21	27.8	26.3	7.2	75	15	10	0.35	>550	1.1	>5500	0.14	0.03	0.4	34	67	280	4.8	<0.01	43	0.1	12	11	0.9	<0.1	0.2	<0.1
21S1345/035	35	28/9/21	23.9	22.1	7.4	78	13	9	0.3	>550	0.9	>5500	0.15	0.01	0.7	41	40	260	4.3	<0.01	39	0.1	20	13	0.9	<0.1	0.2	<0.1
21S1345/036	36	28/9/21	23.5	21.7	7.4	77	12	11	0.25	>550	0.9	>5500	0.14	0.02	0.4	43	46	250	4.3	<0.01	34	0.1	26	12	0.9	<0.1	0.2	<0.1
21S1345/037	37	28/9/21	22.1	19.8	6.9	67.5	16.5	16	0.39	>550	0.8	4800	0.14	0.02	1.6	41	67	270	7.1	<0.01	31	0.1	19	9	1.1	<0.1	0.6	<0.1
21S1345/038	38	28/9/21	22.9	19.9	7.3	74	17	9	0.51	>550	1.1	>5500	0.19	0.02	0.8	48	74	420	5	<0.01	47	0.1	30	17	2.1	<0.1	0.4	<0.1
21S1345/039	39	28/9/21	26.2	24.2	7.4	72	15	13	0.33	>550	1	>5500	0.16	0.02	1.2	44	68	300	3.7	<0.01	39	<0.1	18	13	3.6	0.1	0.4	<0.1
21S1345/040	40	28/9/21	21.8	20	7.4	76	13	11	0.28	>550	0.9	>5500	0.15	0.03	1.3	50	40	290	4.4	<0.01	35	0.1	23	14	1.2	<0.1	0.3	<0.1

APPENDIX J: Box plots for soil sampling data