Identifying wetland soil properties aiding the dormancy of Rift Valley fever vectors in central South Africa

by

Zikhona Gqalaqha

2008104917

Supervisor: Prof C.W. van Huyssteen

Co-supervisor: Dr A.T. Grundling

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Declaration

I, Zikhona Gqalaqha, declare that the Master's Degree research dissertation that I herewith submit for the Master's Degree qualification Master of Science in Soil Science at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

Zikhona Gqalaqha

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Acronyms used

Advanced Very High-Resolution Radiometer sensor ..... AVHRR
Calcium chloride ..... $CaCl_2$
Calcium ..... Ca^{2+}
Cation Exchange Capacity ..... CEC
El Niño southern oscillation ..... ENSO
Electrical conductivity ..... EC
Electromagnetic ..... EM
Farm identity ..... ID
Fluoresce in Diacetate Hydrolysis ..... FDA
Fluorescence ..... XRF
Gravimetric Water Content ..... GWC
Hydrogeomorphic ..... HGM
Inter-tropical convergence zone ..... ITCZ
Magnesium ..... Mg^{2+}
Mean annual precipitation ..... MAP
Mean annual temperature ..... MAT
National Oceanic Atmospheric Administration ..... NOAA
Normalized difference vegetation index ..... NDVI
Polymerase chain reaction ..... PCR
Potassium chloride ..... KCl
Potassium ..... K^+
Reduction potential ..... Eh
Ribonucleic acid ..... RNA
Rift Valley fever virus ..... RVFV
Rift Valley fever ..... RVF
Sodium ..... Na^+
Southern oscillation index ..... SOI
Volumetric water content ..... VWC
Water ..... H_2O
World Reference Base ..... WRB
World Health Organisation ..... WHO
World Organisation for Animal Health ..... OIE
X-ray diffraction ..... XRD

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Abstract

Rift Valley fever (RVF) is a vector-borne zoonotic disease caused by the Rift Valley fever virus (RVFV). This virus is considered one of the largest emerging diseases in Africa and the Middle East, affecting public and livestock health and causing economic loss. RVF causes a high mortality rate in young animals and 100% abortion. The RVF outbreaks are triggered by favourable environmental and flooding conditions, which enable mosquitoes to proliferate and spread the virus. Although environmental factors such as soil, geology and vegetation play a crucial role in the maintenance and survival of the virus, the specifics of these environmental factors are still uncertain and therefore in dire need for more intensive research.

Large epidemics are reported to occur in central South Africa. This study was conducted in the Free State and Northern Cape Provinces of South Africa. The study was initially conducted using 22 selected seasonal and temporary wetland sites (depressions/pans). Of those 22, 12 sites were classified as sites with mortalities and 10 sites were classified as sites without mortalities. The study was subsequently expanded to include eight (8) additional sites, all classified as sites without mortalities.

Soil surveys were conducted: soil classification and soil samples were collected to be subjected to chemical, physical, biological and mineralogical laboratory analysis. Three analyses were performed, which included descriptive analysis, non-parametric van der Waerden test and discriminant analyses. Soil water content was measured at selected sites using level loggers, EM 50, and watermark loggers.

The results from the study indicated that only the cations (exchangeable Ca^{2+}, K^{+}, Mg^{2+}, soluble Ca^{2+} and K^{+}) of all chemical analyses indicated significant differences, with sites without mortalities having higher mean values than sites with mortalities. Coarse silt was the only physical property that differed significantly, with sites without mortalities recording higher mean silt content than sites with mortalities. Coarse silt has a lower water holding capacity than clay and can thus result in drier environments that do not favour mosquito eggs aestivation. Mortality sites and new sites without mortalities were characterised by soils that indicate longer periods of water saturation. This coincided with soil morphological indicators of water saturation, including low chroma colours, presence of mottles and concretions, and lime precipitation.

Ni, Y, Zn and Ba were the only four elements that were found to be significantly different. The first three elements are reported to be toxic and therefore might have suppressed the mosquito eggs aestivation in soils without mortalities. Conversely, Ba was high in the mortality sites.

The microbial activity was inconsistent between the two years of sampling at all sites. The inconsistency was due to various reasons including vastly differing weather conditions

between the two years, possible differing sampling methods and other unknown natural variations.

The hatching of *Aedes* mosquito eggs is promoted by above-normal rainfall that results in flooding of wetlands and thus creates a conducive environment for hatching. In winter months, the hatching of mosquitoes is normally minimal due to lower rainfall, cold temperatures and windy conditions. In some sites, the abundance of mosquitoes was minimal even during flooding, due to 90% of mosquitoes hatching during the first inundation than subsequent flooding.

The discriminant function previously developed was used to classify the (8) new sites without mortalities. Based on this classification, seven sites were erroneously classified as sites with mortalities, while only one site was correctly classified as a site without mortalities. A new discriminant function was subsequently developed using all available data.

The identified RVF outbreak-prone areas coupled with mosquito surveillance and climate data can be used to develop RVF risk maps and thus contribute to the prevention and control of outbreaks. Future research needs to include the collection of *Aedes* mosquito to be tested for the virus, sampling should be expanded to other provinces as this allows for a larger sample to clearly define sites with mortalities and sites without mortalities.

Keywords: discriminant function, RVFV, wetlands, soil properties, soil water saturation

Chapter 1: Introduction

1.1 Background study and motivation

The emergence and re-emergence of infectious diseases are becoming a global concern. Vector-borne viral diseases such as Chikungunya, Dengue fever, Rift Valley fever, Yellow fever and West Nile fever account for over 17% of all infectious diseases globally (World Health Organisation (WHO), 2017). Among these diseases, Rift Valley fever (RVF) is a significant biothreat and biosecurity concern, affecting both livestock and humans. It is spread by some floodwater *Aedes* mosquito species, which have been identified as primary vectors because in nature they maintain and transmit the Rift Valley fever virus (RVFV) transovarially. Secondary vectors include some *Culex species* which intensify early outbreaks by transmitting the RVFV amongst amplifying vertebrate hosts (Linthicum *et al.*, 1984; LaBeaud *et al*., 2010). The virus was first identified in Kenya over 80 years ago. Since then, there have been episodic outbreaks over sub-Saharan Africa and more recently in the Arabian Peninsula (Linthicum *et al*., 2016). According to Gear *et al*. (1951), Mcintosh *et al*. (1980), and Archer *et al.* (2013), South Africa experienced three massive epizootics between the years 1950 to 1951 and 1973 to 1974, with the latest outbreak occurring from 2008 to 2011, impacting the Free State and Northern Cape Provinces. All these epizootics resulted in enormous loss of livestock, pose severe threats to human health and had socioeconomic impacts (Davies *et al*., 1985; Archer *et al*., 2013). Epidemics/epizootics occur at irregular intervals and are highly associated with grassland areas, above-normal rainfall, and sometimes with irrigation systems (Davies, 2010; Linthicum *et al*., 2016).

The ecology of RVF is complex. Infected *Aedes* mosquito eggs are reported (Davies *et al.*, 1985) to survive on the edges of wetlands where the soil dries (i.e. wetlands that are temporary or seasonally wet). It is believed that the eggs hatch every year, however, desiccation resistant eggs allow the RVFV to remain in the soil from season to season. Furthermore, the duration that these resistant eggs remain alive in the soil is poorly understood.

Large epidemics occur at intervals of 5-15 years, depending on the climate of the region. Flooding of *dambos* (*Dambos* are defined as shallow depressions (i.e. hydro-geomorphic wetland type (Ollis *et al*., 2013) referred to as *pans* in South Africa), often located near rivers, which fill with water during the rainy season (Linthicum *et al*., 2016). Horizontal and vertical transmission of the virus and the ecology between the outbreaks are still poorly understood (Linthicum *et al*., 2016). Wetland soil is, however, identified as conducive for the survival of floodwater *Aedes* mosquito eggs that transovarially transmit the virus. Therefore, there is a critical need to investigate wetland soil properties associated with the RVF mortalities.

Research into the estivation of the RVF is a multidisciplinary study that involves the contribution of various disciplines, namely ecology, climate, vegetation, soil, virology and veterinary sciences. This study is part of a broader study with the aim to understand the occurrence and prevalence of Rift Valley fever in South Africa. However, there is a dearth of knowledge relating to soil research. Historically, only one study has examined the relationship between wetland soil properties and the RVF epidemics (Verster, 2016). Verster (2016), conducted a study that aimed to understand the relationship between wetland soil properties and the occurrence of RVF. The analysis identified eight (8) soil properties that were associated with the occurrence of livestock mortality in the 2010 RVFV outbreaks. These properties include soluble calcium (Ca^{2+}), exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), cation exchange capacity (CEC), medium sand, arsenic (As) and bromide(Br).

The first six (6) variables had higher concentration values in soils of sites with mortalities compared to sites without mortalities. As and Br concentrations, however, were lower at mortality sites compared to sites without mortality. Bobwell *et al*. (2014) reported that As acts as a toxic element in the soil environment. It is suspected that high As levels could suppress the virus at sites without mortalities. This research expands on the study of Verster in (2016), by including eight additional sites, supplementary soil analysis and soil water content measurements.

The current study was conducted over 22 sites and expanded to include eight additional sites, based on a cross-sectional survey of the farms that had seropositive sheep (i.e. testing positive for the presence of a virus) but were never vaccinated. Additional analyses were conducted to examine the correlation between the occurrence of RVF and non-agronomic soil properties as well as soil water content. It is anticipated that all these efforts will aid in understanding the provenance of the RVFV.

1.2 Objectives

The objectives of this study were:

- To evaluate wetland soil properties that best relate to the occurrence and absence of RVF vectors (*Aedes* and *Culex*) based on the 2010 outbreak classification.
- To determine seasonal microbial activity at the sites in relation to soil water levels, mosquito abundance and species.
- To validate the existing discriminant function for predicting sites with RVF-associated mortalities.

Chapter 2: Literature review

2.1 Wetlands

The term wetland is defined differently all over the world, with over 50 different definitions of wetlands currently in use (Dugan, 1990). Cowardin *et al*. (1979) noted that, there is no universally agreed definition and classification of wetlands, and that they differ between countries. McCartney *et al*. (2010) supported this statement and highlighted that the differences in wetland definitions result in colossal confusion and uncertainty about the number of wetlands worldwide. However, there seems to be a scientific agreement that wetlands cover approximately 6% of the earth's surface (Inglett *et al*., 2005; McCartney *et al*., 2010). In South Africa, the National Water Act defines a wetland *"as the land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil"* (National Water Act, 1998). The area may be saturated with water permanently (12 months waterlogged), seasonally (5-11 months waterlogged) or temporarily (1-5 months waterlogged), which then supports hydrophytic plants and animals, adapted to life in saturated soils (Brown & Cook, 2014). The hydrological zones will be further discussed in section 2.1.2. The most widely accepted wetland definition is by the Ramsar Convention of 2008. Over 100 countries signed and accepted the definition where they defined wetlands as "*areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres*" (The Ramsar Convention Manual, 2008).

2.2 Wetland importance and functions

For many years, the importance of the wetland ecosystem has been overlooked, which has resulted in more than half of the world's wetlands being degraded (Schuyt, 2005). Wetlands are considered by many to have little or no value (Brander & Schuyt, 2010). Another possible explanation of why wetlands are considered wastelands is the lack of awareness and relevant knowledge on the advantages that wetlands provide to the community and the world at large. Wetlands provide ecosystem services, either directly or indirectly. Indirect contributions include providing habitat and food for diverse species, purifying water thus improving water quality, trapping pollutants such as sediments and heavy metals, mitigating floods and drought, recharging groundwater, streamflow regulation, phosphate assimilation, nitrate assimilation, erosion control, and carbon storage, *etc.* (Kotze *et al*., 2009). The direct

contributions include water supply, provision of harvestable resources, provision of cultivated foods, tourism, recreation, cultural heritage education and research (Mitch & Gosselink, 2000).

2.2.1 Types of wetlands and hydrological zones

There are many different types of wetlands in the ecosystem which are classified accordingly into different groups. In South Africa, wetland types are classified according to the concept of hydrogeomorphic (HGM) units (Kotze *et al*., 1994; Ewart Smith *et al*., 2006). This classification system includes depressions (*pans*), seepage wetlands, un-channelled valley bottoms, channelled valley bottoms and floodplains (Kotze, 1999; Ollis *et al*., 2013).

Due to the inconsistency of the slope, soil type, climate, and vegetation the hydrological regime varies throughout the wetland, resulting in different zones of wetness, e.g. temporary, seasonal and permanent zones (Figure 1; Kotze, 1996).

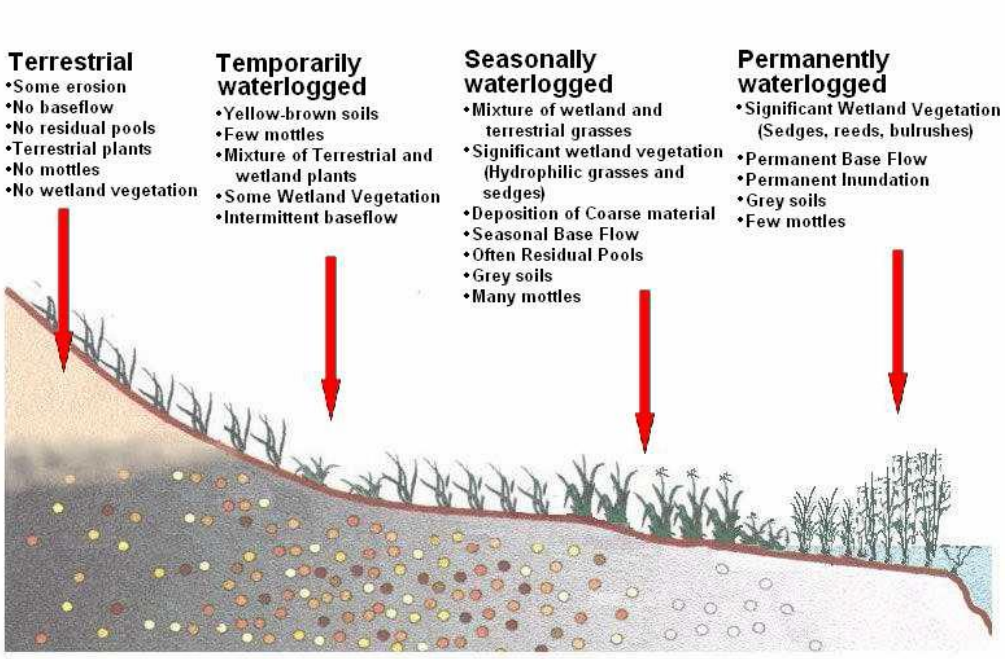


Figure 1 A cross-section through a wetland that depicts how soil wetness and vegetation changes from the upland, down through the wetland (adapted from Kotze, 1996).

2.3 Wetland delineation

There are four important indicators used to delineate wetlands. These include hydrology, hydrophytic vegetation, hydric soils and landscape setting (Braack *et al*., 2000; DWAF, 2005). There is a close relationship between these indicators. They are interdependent of hydrology, which is the only independent variable (US Army Corps of Engineers, 1987; Tiner, 1993). Hydrology and hydrophytic vegetation will be discussed briefly in the following paragraphs, while hydric soils will be discussed in detail thereafter.

2.3.1 Wetland hydrology

Hydrology is the main factor used to determine the presence or absence of wetlands and arguably creates and maintains all wetlands (Tiner, 1993; Hurt & Carlisle, 2001; Council Greater Wellington Regional, 2005). Hydrology supports wetland structure and functions and as well as the productivity of the wetland (Council Greater Wellington Regional, 2005). Mottling and colour (low chroma) are all determined by hydrology and these are the important indicators of wetland hydrology used for wetland delineation (National Research Council, 1995; DWAF, 2005).

2.3.2 Hydrophytic vegetation

Wetland vegetation (hydrophytes) is the most visible indicator used for wetland delineation and the most likely to grow in water or wet areas. The type of vegetation is driven by the hydrology, which creates a diverse set of environmental conditions with different degrees of wetness allowing for different vegetation species to adapt to the conditions (Tiner, 1993). Vegetation indicator categories were developed to distinguish species according to their association with degree of wetness (Table 1).

Table 1 Vegetation grouped according to wetness tolerance (US Army Corps of Engineers, 1987; Tiner, 1999)

Hydric status	Description/Occurrence
Obligate wetlands species (OBL)	99% of the time these plants occur in wetlands under natural conditions. Example: *Spartina alterniflora*
Facultative wetland species (FAWC)	These plants are usually found in wetlands (67-99%), but can rarely be found in uplands. Example: *Cornus stolonifera*
Facultative species (FAC)	These species grow both in wetlands and non-wetlands. Example: *Gleditsia triacanthos*
Facultative upland species (FACU)	Most of the time these species do not occur in wetlands, but can sometimes be found in wetlands. Example: *Quercus rubra*
Obligate upland species (UPL)	These species almost always occur in non-wetland areas (99%). Example: *Pinus echinata*

2.3.3 Hydric soils

Generally, the soil is formed through five factors, namely topography, climate, parent material, organisms, and time (Jenny, 1994). Wetland soils are widely distributed throughout the world and they vary depending on the hydrological regimes, climatic conditions, soil formation processes and geomorphological settings. Hydric soils support hydrophytic vegetation (US Army Corps of Engineers, 1987; Tiner, 1993; Inglett *et al*., 2005). Various scientists have defined hydric soils differently, for example, Vepraskas (2001) defines hydric soils as soils that formed through oxidation and reduction chemical reactions that occur under anaerobic soil conditions. Similarly, the US Army Corps of Engineers (1987; 2012) and Hurt & Carlisle (2001)

defined hydric soils as soils formed under conditions of water saturation, flooding or in areas where water stands for a long time. It is through this process that roots and microorganisms slowly consume all of the oxygen present in pore spaces in the soil during the growing season, causing the development of anaerobic soil conditions. According to Amore *et al*. (1985), hydric soils are formed through continuous periods of water saturation where the accumulation of organic carbon and mild temperatures play a significant role in facilitating reductive dissolution and segregation of iron (Fe) and manganese (Mn) into redox concentrations and depletions. Therefore, one can conclude that for a hydric soil to form, there should be poorly drained soils that lead to the occurrence of free standing water. It is these anaerobic soil conditions that result in the reduction of Fe, Mn and sulphate (SO_4^{2-}), which govern plant growth and the accumulation of organic matter. All the above-mentioned processes make it easy to identify hydric soils in both dry and wet seasons (US Army Corps of Engineers, 2012). It is essential to note that hydric soils are not only found in permanent wetland areas but can also be found in intertidal zones. Conversely, there is a possibility that hydric soils might not develop in all wetlands (Lewis *et al*., 1995).

For field identification, soil colour is the main criterion used to identify hydric soils (Lewis *et al*., 1995). US Army Corps of Engineers (1987), stated that soils commonly found in permanent wetlands are Histosols, Peat and Muck, while Folists are found towards the drier hydrological spectrum. These soils develop due to changes in hydrological regimes, for instance, these include in swamps, bogs and marshes which are always saturated, riparian systems which are saturated for short periods and groundwater recharge areas (Vepraskas & Faulkner, 2001).

2.3.3.1 Redox reactions and development of redoximorphic features

During redox reactions, electrons are transferred amongst atoms. Atoms that have less than four electrons in the outer shell usually donate electrons and increase in valence. Conversely, atoms that contain more than four electrons tend to accept electrons to fill the outer shell and decrease in valence (Vepraskas & Faulkner, 2001). Scientists like Vepraskas & Faulkner (2001) and Szogi *et al.* (2004), indicate that oxidation and reduction reactions must take place simultaneously. Loss of electrons by atoms is referred to as oxidation. Examples of elements that undergo oxidation include sodium (Na), magnesium (Mg) and calcium (Ca), *etc*. On the other hand, the gain of electrons by atoms is termed as reduction, e.g. nitrogen (N), carbon(C) and oxygen (O_2). Hydrogen (H) is an exception that has only one electron; therefore, it can either donate or accept an electron. A complete redox-reaction must contain at least an oxidation and a reduction component, each of which is called a half-reaction (Table 2; Mitch & Gosselink, 2000). Each redox reaction has different effects on the soil. According to McBride

(1994), Fe^{2+} and Mn^{2+} can be available in the soil even if oxygen is not fully depleted. This can be due to non-equilibrium of electrons and half-reactions within the soil.

Table 2 Half reactions of redox reaction occurring in the soil (Mitch & Gosselink, 2000)

Chemical element	Reduction	Reduction reaction (gain of electrons)	
Oxygen	O_2 H_2O	$O^{2-} + 4e^- + 4\ H^+$	$\leftrightarrow 2\ H_2O$
Nitrogen	NO_3^- N_2O, N_2	$2\ NO_3^- + 10\ e^- + 12\ H^+$	$\leftrightarrow N_2 + 6\ H_2O$
Manganese	Mn^{4+} Mn^{2+}	$MnO_2 + 2\ e^- + 4\ H^+$	$\leftrightarrow Mn^{2+} + 2\ H_2O$
Iron	Fe^{3+} Fe^{2+}	$Fe_2O_3 + 2\ e^- + 6\ H^+$	$\leftrightarrow 2Fe^{2+} + 3\ H_2O$
Sulphur	SO_4^{2-} H_2S	$SO_4^{2-} + 8\ e^- + 10\ H^+$	$\leftrightarrow H_2S + 4\ H_2O$
Carbon	CO_2 CH_4	$CO_2 + 8\ e^- + 8\ H^+$	$\leftrightarrow CH_4 + H_2O$
Hydrogen	H^+ H_2	$2\ H^+ + 2\ e^-$	$\leftrightarrow H_2$

During redox reactions, oxidised compounds are reduced to the elements that are shown in Table 3 below. Redox reactions are driven by hydrology, oxidation of organic matter and microorganisms (bacteria & fungi) that are responsible for the decomposition of organic matter. Different atoms act as electron acceptors in these reactions. For example, in an aerobic environment, oxygen acts as the dominant electron acceptor. Soil microbes favour oxygen since it produces the most energy during respiration (Vepraskas & Faulkner, 2001). In seasonal wetlands, oxygen becomes less available due to the soil being saturated. In the permanent wetland zone where anaerobic wetlands are dominant, all the available oxygen becomes reduced. Since the conditions are no longer favourable for aerobic respiration, the bacteria have to find alternative electron acceptors to utilise to maintain their metabolism and produce energy. These electron acceptors include NO_2^-, MnO_2, $Fe(OH)_3$, SO_4^{2-} and CO_2, which are reduced in the sequence given above. Simply put, when conditions are no longer favourable for the oxygen to be utilised, NO^-_3 is the first acceptor to be used. Once all the NO_3^- is reduced, MnO_2 is used, followed by $Fe(OH)_3$, SO_4^{2-} and finally CO_2 (Vepraskas & Faulkner, 2001; Szogi *et al*., 2004; Fiedler *et al*., 2007; Smith & Van Huyssteen, 2011). Vepraskas (1995), reports that a site is considered a wetland only once Fe^{3+} is reduced to Fe^{2+}. Sparks (2003), suggested that as NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-} and CO_2 are getting reduced in the soil, the reduction potential (Eh) which measures the electron (e^-) activities is also decreasing. However, the pH which measures the (H^+) activities shows an inverse relationship and is increasing (Table 3).

Table 3 Redox reaction of elements (Mitch & Gosselink, 2000; Inglett *et al*., 2005)

Chemical element	Oxidised form	Reduced form
Oxygen	O_2 (Oxygen)	H_2O (Water)
Nitrogen	NO_3^- (Nitrate)	N_2O, N_2
Manganese	Mn^{2+} (Manganic)	Mn^{2+} (Manganous)
Iron	Fe^{3+} (Ferric)	Fe^{2+} (Ferrous)
Sulphur	SO_4^{2-} (Sulphate)	H_2S (Sulphide)
Carbon	CO_2 (Carbon dioxide)	CH_4 (Methane)
Water	H_2O (Water)	H_2 (Hydrogen)

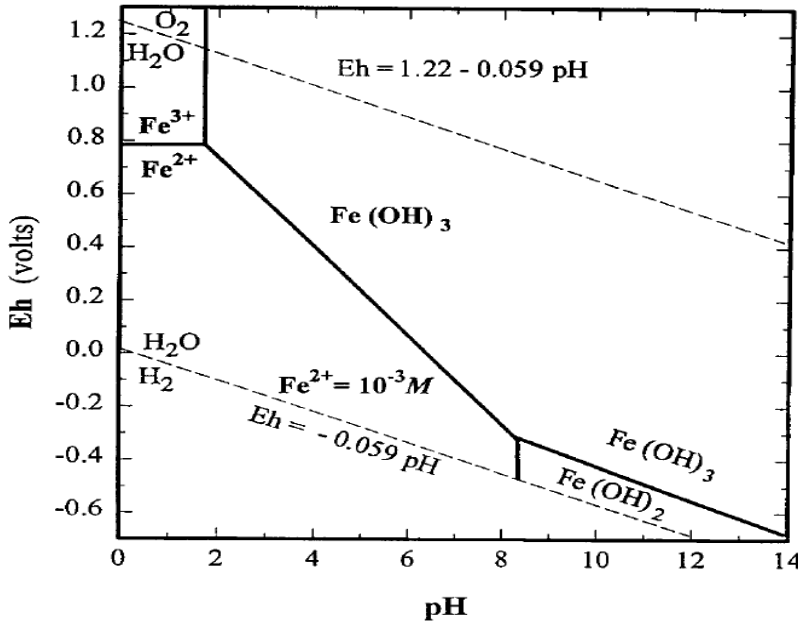


Figure 2 The relationship between Eh and pH and iron species in soil (Sparks, 2003).

2.3.3.2 Redoximorphic features and hydric soil field indicators

In a wetland environment, redoximorphic features and hydric soil field indicators demonstrate that reduction and oxidation reactions have occurred (Vepraskas, 2001). According to Vepraskas *et al*. (1999), there are three field indicators for hydric conditions. These conditions include the reduction of Mn and Fe, accumulation of organic carbon and the reduction of SO_4^{2-} to H_2S gas. In comparison, Amore *et al*. (2004) reported that the three main requirements for the formation of redox features in the soil include prolonged periods of water saturation, accumulation of organic carbon and warm temperatures that are responsible for speeding up soil microbial activities and lastly there must be enough Fe and Mn in the soil. The effects of these conditions include changes in colour, thickness, and depth of the soil. It is important to highlight that Mn is always reduced before Fe in saturated soils and that the reduction of Fe will not take place if Mn is not depleted (Vepraskas *et al*., 1999). Vepraskas *et al*. (1999) and Vepraskas *et al*. (2006), stated that it can take roughly seven years for redox features to form within the soil provided that the soil stays saturated for at least three months in each year. In contrast, Fiedler *et al*. (2007) reported that hydric field indicators could form in 3 years or even less if there are continuous wet and dry cycles.

Research by Van Huyssteen *et al*. (2005), Le Roux *et al*. (2010) and Van Huyssteen *et al*. (2010) indicates that reduction starts when the soil is 70% saturated with water. Smith & Van Huyssteen (2011), conducted a study based on the duration and different degrees of water saturation. The experiments were performed over four treatments to varying degrees of water saturation (i.e. 60%, 70%, 80% and 90%) over 121 days. During this period, it was found that at 60% and 70% saturation the soil was still aerobic. Reduction only took place when the soil was 80% and 90% saturated but was more significant at 90% saturation (Smith & Van Huyssteen, 2011). Therefore, it was concluded that a significant reduction started between 70% and 80% and was highest at 90%. Furthermore, it was reported that Mn^{2+} and Fe^{2+} were responding positively to the increase in water saturation as shown when an increase in water saturation resulted in Mn^{2+} and Fe^{2+} concentration increasing with time. It is, however, crucial to mention that scientists have not determined how long it takes precisely for hydric field indicators to form in the soil and there are inadequate studies concerning this (Vepraskas, 1999). Smith & Van Huyssteen (2011), proposed further studies based on this concept.

2.3.3.3 Oxidation-reduction potential (Eh) in wetland soils

The oxidation-reduction potential (Eh) measures the number of free electrons exchanged during redox reactions. The Eh in wetland soils ranges between +700 mV to -300 mV (Reddy *et al*., 2000). The positive value represents low electron activity whereas the negative value represents the high electron activity. It is said that the fluctuating water table in wetland environments results in fluctuating Eh. Areas, where Eh is above +400 mV (temporary wet zone), are considered aerobic, i.e. oxygen is still available and organic matter is rapidly decomposed. Between an Eh of +200 and 0 mV (seasonal wet zone), the environment is becoming poor in oxygen, the soil is slowly becoming anaerobic and the decomposition of organic matter is slowed down. In an environment where the Eh is between 0 and -200 mV (permanent wet zone), the soil is anoxic and electron acceptors are starting to be reduced. Thus Mn^{4+} is reduced to Mn^{2+} and Fe^{3+} is reduced to Fe^{2+} (Table 4; Reddy *et al*., 2000). It is, however, crucial to highlight that when Mn and Fe are oxidised they are insoluble and immobile in the soil. In contrast, when these elements are reduced, they are soluble and are consequently mobile, meaning they can easily be leached out from the soil (Vepraskas, 2001). If anaerobic conditions prevail for prolonged periods the Eh will continue to fall. Once it reaches an Eh between -200 to -300 mV then all Fe and Mn will be reduced, allowing SO_4^{2-} to become the next element to be reduced by microbes (Reddy *et al*., 2000; Vepraskas, 2001; Inglett *et al*., 2005). Upon reduction of SO_4^{2-}, H_2S gas is released, which smells like a rotten egg and the soil becomes dark black. This characteristic makes it easy to identify hydric soils (Hurt & Carlisle, 2001). McBride (1994) states that if anaerobic conditions prevail for long, i.e. until the Eh becomes less than -200 mV, then CO_2 will undergo reduction. This results in the

production of methane, seen as bubbles rising to the water surface (McBride, 1994; Vepraskas & Faulkner, 2001).

Table 4 Relationship between soil saturation and redox potential (Reddy *et al*., 2000)

AEROBIC				Soil Condition
ANAEROBIC				
Highly Reduced	Reduced	Moderately Reduced	Oxidised	Redox Condition
CO_2	SO_4^{2-}	$Fe^{3+}Mn^4NO^-_3$	O_2	Electron Acceptor
Anaerobic		Facultative	Aerobic	Microbial Metabolism
-200		0 +200 +400 +600		
Redox Potential (Eh)-Millivolts				

As soon as the dry season approaches, the soil slowly becomes unsaturated. Oxygen re-enters the soil, the Eh increases and the last element to be reduced is the first to be oxidised. The H_2S gas becomes the first to be depleted, Fe^{2+} oxidises and then precipitates, leaving the grey colours in the soil. This usually happens in permanent wetlands. In seasonal and temporary wetlands where the water table is fluctuating, Mn and Fe precipitate upon oxidation, resulting in the formation of mottles (Table 5). Black mottles indicate oxidised Mn, while red, yellow, yellow-brown and orange mottles indicate oxidised Fe (Vepraskas, 2001).

Table 5 Soil mottle mineralogy and colour in relation to the oxidation state of Mn and Fe (Sposito, 1989)

Mineral name	Formula	Colour (Munsell)
Hematite	α-Fe_2O_3	5R-2.5 YR(Bright red)
Maghemite	γ-Fe_2O_3	Reddish-brown
Magnetite	Fe_3O_4	Black
Goethite	α-FeOOH	7.5YR-10YR (Yellowish-brown)
Lepidocrocite	γ-FeOOH	5YR-7.5YR (Orange)
Ferrihydrite	$Fe_5HO_3•4H_2O.Fe_5(O_4H_3)_3$	5YR-7.5YR (Reddish Brown)
Green Rust	$Fe^{2+}Fe^{3+}$ (Hydroxy compound)	Greenish Blue

2.4 Rift Valley fever virus

Rift Valley fever (RVF) is a mosquito-borne viral zoonotic disease transmitted from animals to humans (Aradaib *et al*., 2013). It is caused by the Rift Valley fever virus (RVFV) that belongs to the *Phenuiviridae* family. This is a family of Ribonucleic acid (RNA) viruses and the *Phlebovirus* genus (Bishop *et al*., 1980; Gerdes, 2004; Pepin *et al*., 2010; Ikegami & Makino, 2011; Linthicum *et al*., 2016; Maes *et al*., 2018). RVFV causes disease not only affecting domestic animals such as sheep, cattle and goats but also wild animals such as buffalo, camel, zebra, elephant, warthog, impala and waterbuck. Sheep are known to be the primary amplifying host amongst all the above-mentioned animals (Davies *et al*., 1975). Pregnant and young animals are the most susceptible to this disease. Epizootics are characterised by mass abortions among pregnant ewes and a high mortality rate in new-borns, estimated at 100% (Davies, 2010). Adult animals may suffer from nasal discharge, weakness and a decrease in

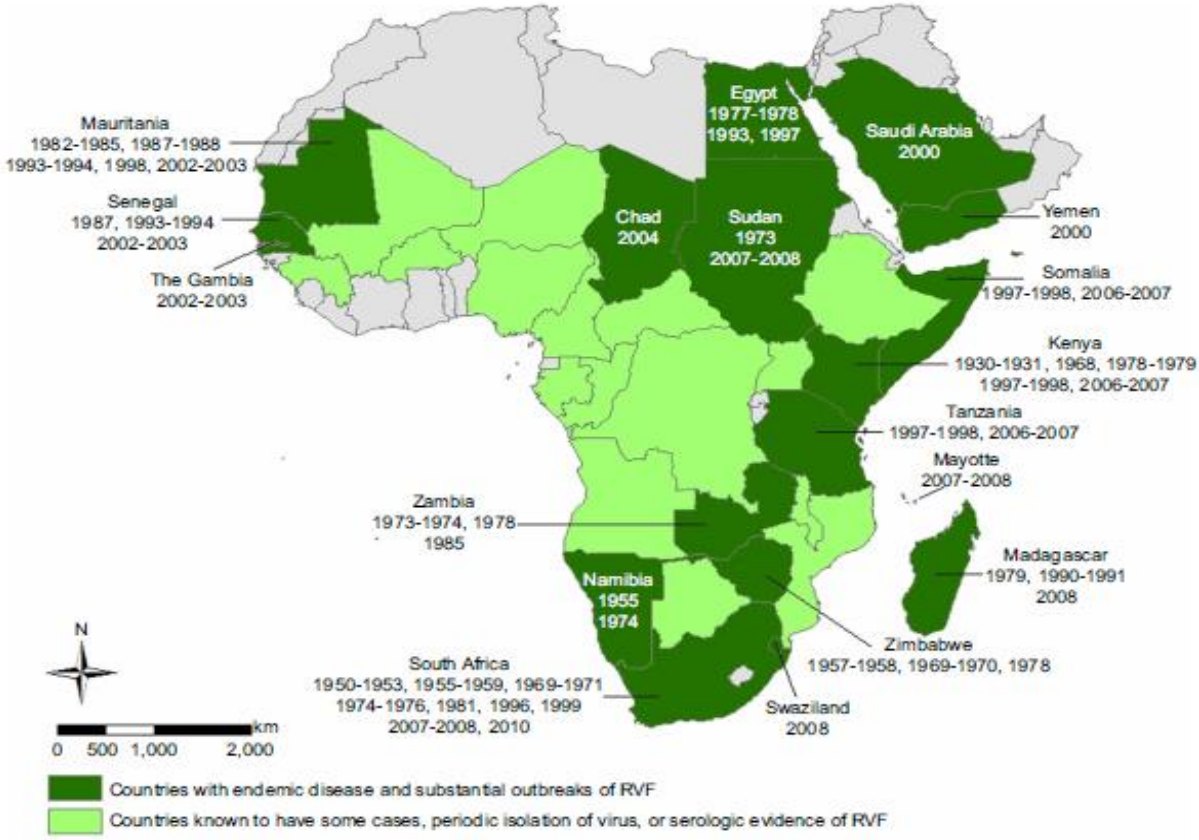
milk production (Shawky, 2000). The mortality rate is approximately 30% in adult animals and 100% in young animals (Balkhy & Memish, 2003; Linthicum *et al*., 2016).

RVF may also infect humans through direct contact with infected tissues and fluids and in rare cases can be transmitted through a mosquito bite (Balkhy & Memish, 2003; Anyamba *et al*., 2012). However, human-to-human transmission has not been detected so far. People working in butcheries, farmers and veterinarians are more likely to be exposed to RVFV (Linthicum *et al*., 2016). Typical symptoms in humans include febrile diseases, with 1% of these cases developing into severe diseases such as haemorrhagic fever, vision loss, acute hepatitis, encephalitis, and retinitis and can lead to death in rare cases (Turell *et al*., 2008; Ikegami & Makino, 2011; Aradaib, 2013).

2.4.1 Geographic distribution

RVF is limited to African countries and Saudi Arabia (Linthicum *et al*., 2016). The first outbreak of this disease was detected in the Great Rift Valley in Kenya during the 1930s (Daubney *et al.*, 1931). However, it is suspected that the RVF outbreaks occurred before 1930 due to a report released by Stordy in 1913 that described comparable medical signs and symptoms that correlate with RVF (Davies, 2010). Outbreaks occurred in Kenya in 1931, 1968, 1978 to1979, 1997 to 1998 and 2006 to 2007 (Swanepoel & Coetzer, 2004). Figure 3 indicates countries in Africa that have had widespread RVF outbreaks. These include Tanzania, Somalia, Egypt, Sudan, Chad, Senegal, Gambia, Mauritania, Mozambique, Zambia, Zimbabwe, Namibia and South Africa (Swanepoel & Coetzer, 2004; Rolin, 2013). Although antibodies to the diseases were found in animals as well as humans in countries like Angola, Chad, Gabon and Nigeria, these countries have not reported RVF infections (Gerdes, 2004). In 1979, RVF was identified in Madagascar and 11 years later from 1990 to 1991, resulted in large outbreaks within the country (Linthicum *et al*., 2016). In 2000, RVF was diagnosed as the cause of an acute epizootic in Saudi Arabia and Yemen, this was the first time RVFV occurred outside Africa. Several livestock mortalities followed these outbreaks and human cases were also reported (Martin *et al*., 2008; Linthicum *et al*., 2016). Davies (2010) suggests that RVF epizootics in Saudi Arabia occurred in the ecological environment that is similar to African countries that are endemic to RVF.

Sissoko *et al*. (2009) reported that RVF was discovered in Mayotte in 2007 for the first time. To date, RVF has been found in more than 30 countries and it seems possible for the virus to spread worldwide through vectors that can transmit the virus because of animal trading and human movements (GF-TADs, 2012).



The dark green colour indicates countries that are endemic to RVF and light green colour indicates countries with some cases, periodic isolation of virus and serological evidence of RVF with the years of the occurrences

Figure 3 Geographical distribution of countries affected by RVFV since 1930 in Africa (Rolin, 2013).

2.4.2 Rift Valley fever outbreaks in South Africa

South Africa experienced three significant outbreaks of RVF. The first outbreak was recorded between 1950 and 1951, lasting approximately five months. This outbreak started in December 1950 and continued until April 1951. Geographically, the Free State, Northern Cape and Eastern Cape Provinces were the most affected by these epizootics (Figure 4; Archer *et al*., 2013). Small towns of the Western Free State (i.e. Dealesville, Bultfontein, Brandfort and some zones of the Boshof district and Koffiefontein) hosted most of these epizootics (Thompson & Pienaar, 2013). The results reported from these outbreaks include 100 000 sheep deaths and 500 000 abortions in pregnant sheep (Swanepoel & Coetzer, 2004; Martin *et al*., 2008; Thompson & Pienaar 2013; Linthicum *et al*., 2016).

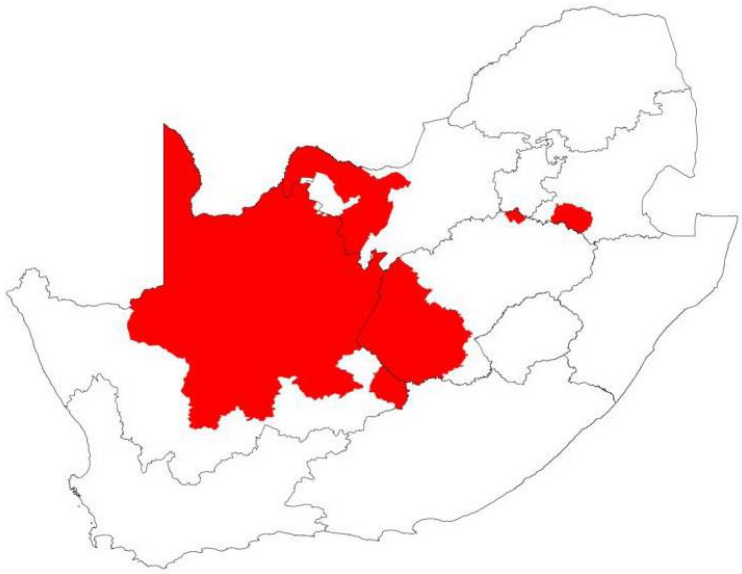


Figure 4 Affected areas of RVF outbreaks in South Africa during 1950 and1951 (Thompson & Pienaar, 2013).

Between 1973 and 1974, South Africa experienced the second intensive RVF outbreak. The outbreak started in the Free State in Bulfontein and rapidly spread throughout the entire Free State Province. The epidemic extended to neighbouring provinces, that is, the Northern Cape, Eastern Cape, North West, parts of Gauteng, KwaZulu-Natal and to some parts of Western Cape (Figure 5; Coetzer, 1977; Thompson & Pienaar, 2013). This outbreak resulted in a mortality rate of approximately 95% in lambs and 15-20% in adult sheep (Coetzer, 1977).

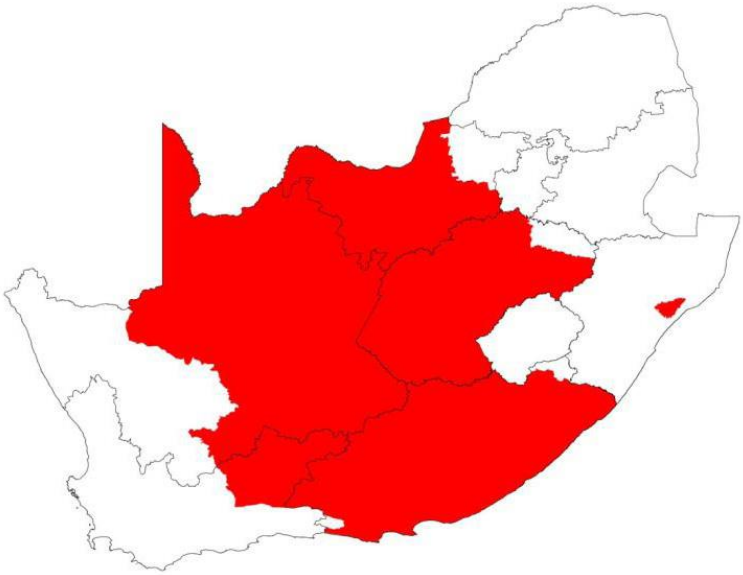


Figure 5 The extent of the RVF outbreak during 1973 and 1974 (Thompson & Pienaar, 2013).

The next outbreak emerged between 2008 and 2011. In 2008 and 2009, the RVF outbreaks were smaller. During this period, the Mpumalanga, Limpopo, Gauteng, Eastern Cape and North West Provinces were mostly affected (Archer *et al*., 2011; Thompson & Pienaar, 2013).

From 2010 to 2011 a more extensive outbreak occurred. This outbreak was so intensive that it almost affected the entire country and recorded a high mortality rate, shown in Figure 6. Thompson & Pienaar (2013) state that it was the first time in the history of RVF in South Africa that an outbreak occurred during winter.

The most recent outbreak occurred in May 2018, at Salobe Farm in Jacopsdal Free State Province. The outbreak followed the trend of the previously reported outbreaks where it re-emerged after increased rainfall following drought periods. During this recent outbreak, 250 of the 600 sheep aborted (van Vuren *et al*., 2018). Six (6) people were reported to show symptoms of RVFV after handling carcasses of animals that aborted lambs but tested negative from polymerase chain reaction (PCR) (van Vuren *et al*., 2018; OIE Rift Valley fever South Africa, 2018). It is important to note that since 1950, small outbreaks were also reported in between these major outbreaks (Archer *et al*., 2013).

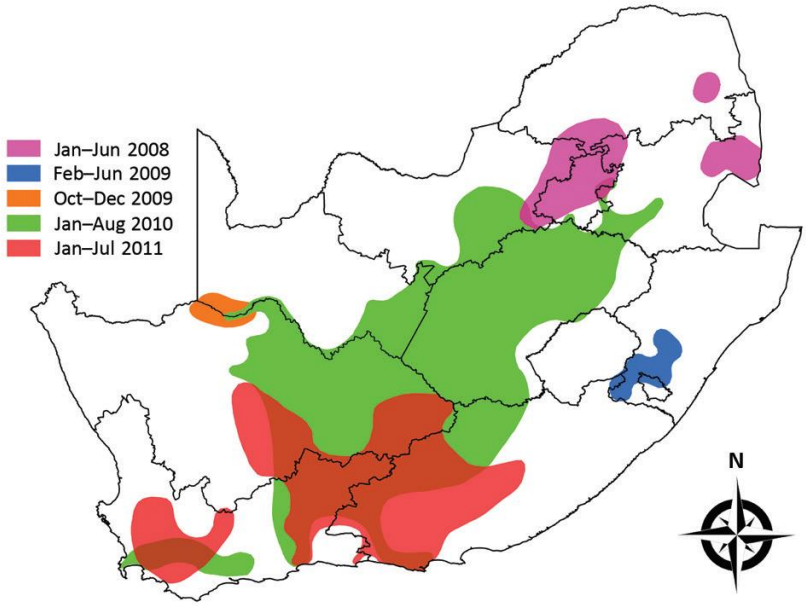


Figure 6 Occurrence of RVF epidemics from 2008 to 2011 in five regions of South Africa. The colours indicate the temporal history of outbreaks in different regions (Williams *et al*., 2016).

2.4.3 Ecology of Rift Valley fever virus

Across the African continent and the Middle East, RVFV has been reported to be a huge scourge and major research area. There is, however, a huge gap in some of the ecological aspects of the virus. The ecological aspects remain poorly understood, especially regarding how it plays a role in the maintenance and survival of the virus and the mosquito eggs during the estivation period. Amongst all the countries that have recorded RVF, the ecology plays a pivotal role in the ability to sustain the RVFV under different environmental conditions, resulting in epidemics (Njenga & Bett, 2019). Therefore, the geographic distribution of RVF

epidemics can be predicted based on the environmental conditions of sites where known occurrences of RVF have been observed (Ochieng, *et al*., 2016).

Climatic factors such as rainfall have been well documented relating to how it affects the RVF endemics. However, other factors such as altitude, vegetation, geological and geographical factors (including soil properties) are yet to be further investigated (Njenga & Bett, 2019; Hightower *et al.*, 2012). In the study conducted by Munyua *et al.* (2016), it was highlighted that soil types are one of the most important soil properties to maintain and support RVFV. Previous investigations speculated that certain soil types (including Solonetz, Calcisols, Solonchaks and Planosols) were highly associated with RVF during Kenyan outbreaks of 2006/2007 (Hightower *et al*., 2012). Another study conducted by Munyua *et al*. (2016) indicated that Vertisols, Solonertz and Luvisols also increase the risk of RVF epidemics when compared to other soil types. On the other hand, Njenga & Bett (2019) included Nitisols as another soil type associated with a high risk of RVF epidemics. These soil types are positively associated with a high risk of RVF epidemics due to their poor drainage properties, retaining water better than other soil types, saturating more rapidly and also remaining saturated for longer (Hightower *et al*., 2012; Munyua *et al*., 2016; Njenga & Bett, 2019). Notably, RVF epidemics are not only associated with the above-mentioned soil types, but also with other soil types that are characterised by high clay content, with poor drainage and that can retain water for longer periods (Hightower *et al*., 2012).

2.4.4 Rift Valley fever transmission

Mosquitoes, especially the floodwater mosquito species, seem to be the primary host for transmitting RVFV and are responsible for sustaining this virus from egg to adult. Secondary vectors of *Culex, Mansonia* and *Anopheles* species are associated with the rapid spread of RVF, leading to large epidemics (Taylor *et al*., 2016). It is important to note that mosquito vectors that transmit this disease in eastern parts of Africa as well as in South Africa differ from those of West Africa (Balkhy & Memish, 2003). The common *Aedes* mosquito vectors in South and East Africa include *Aedes circumluteeolus* and *Aedes micintoshi* while common vectors in West Africa include *Aedes vexans* and *Aedes ochraceus* (Balkhy & Memish, 2003).

2.4.5 Rift Valley fever and climate

1. Changes in global climate anomalies can cause drastic changes in rainfall, humidity and temperature which may result in flooding or drought (Anyamba *et al*., 2012). These climatic patterns are considered as the primary drivers that contribute to outbreaks of vector-borne diseases (Mirski *et al*., 2011; Anyamba *et al*., 2012). These climate primary drivers can activate and enhance the survival of vector-borne diseases by creating suitable habitats for vectors, proliferating population numbers of these

arthropods and producing favourable conditions for them to spread (Mirski *et al*., 2011; Anyamba *et al*., 2012). For example, the occurrence of RVF outbreaks is associated with heavy rains (Mason, 2001; Anyamba *et al*., 2009).Heavy rains could be a result of different climatic conditions such as the development of a strong inter-tropical convergence zone (ITCZ). The ITCZ is a zone near the equator where northern and southern trade winds come together, converge and result in large amounts of precipitation (Davies *et al*., 1992; Linthicum *et al*., 1999).

2. El Niño southern oscillation (ENSO) events have a huge impact on climate and weather anomalies, which may lead to floods or drought events (McPhaden *et al*., 2006). RVF for example, is associated with warm ENSO in East Africa, which is linked to above-normal precipitation and mild/cooler temperatures, whereas in southern Africa, heavy rainfall is associated with La Niña (Anyamba, *et al*., 2009; Anyamba *et al*., 2010). The commonly used index to represent different phases of ENSO and predict above-average rainfall is the southern oscillation index (SOI) (Linthicum *et al*., 1999). Manifestations of negative SOI phases are above normal rainfall in East Africa and below-normal rainfall in Southern Africa, whereas the positive phase of SOI results in below normal rainfall in East Africa and above normal rainfall in Southern Africa. Figure 7 shows a comparison between RVF outbreaks and the SOI anomalies in Kenya from 1950 to 1998. During this period, there were eight outbreaks of RVF where the SOI anomalies were two or more (Linthicum *et al*., 1999).

Depending on the climate of the region, RVF is reported to occur at irregular intervals of 5 to 15 years and 15 to 30 years (Davies *et al*., 1985). For example, in areas that receive less rainfall and are more prone to drought in the South and East Africa, RVF and other Savanna areas epizootics occur at intervals of 5 to 15 years following continuous rain. Correspondingly, epizootics may have a longer interepidemic period, 15 to 30 years in areas that receive much less rainfall (Davies *et al*., 1985).

During the period from 1950 to 1998, eight RVF activities and 13 periods when there were strong negative anomalies in the SOI were recorded indicated by the dates and the bars in the graph (Figure 7; Linthicum *et al*., 1999).

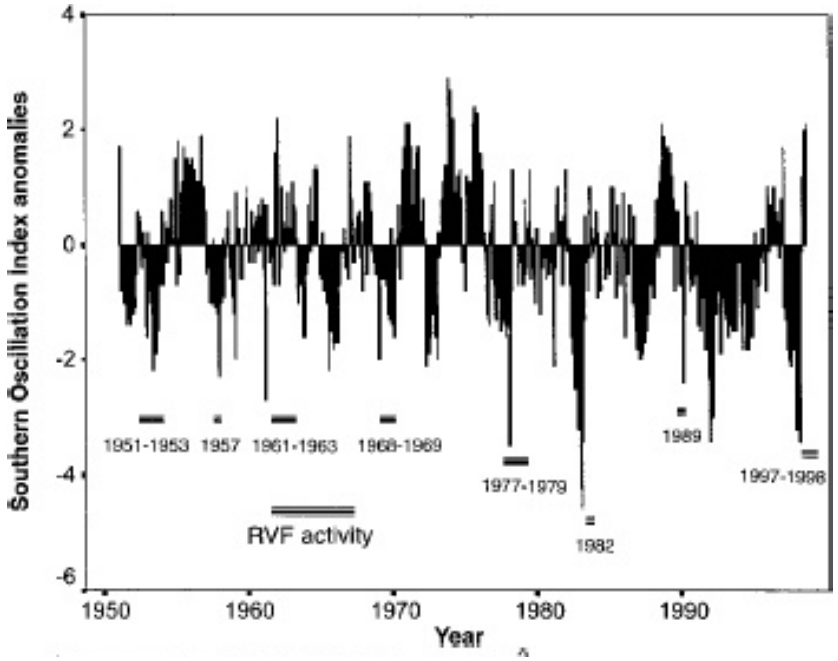


Figure 7 Comparison of SOI and RVF outbreaks over a period of 48 years (1950 to 1998). During this period eight RVF activities and 13 periods when there were strong negative anomalies in the SOI were recorded indicated by the dates and the bars in the graph (Linthicum *et al*., 1999).

2.4.6 Remote sensing and the prediction of Rift Valley Fever

There is general agreement in the literature that climate fluctuations due to ENSO events lead to increased rainfalls followed by an intense drought period associated with large epidemics/epizootics. It is these large epidemics that attract the attention of most of the countries that are endemic to RVF. However, it is believed that there are always minor outbreaks occurring in between these years, as was identified by the serological tests, which measure the RVF viral antibodies on tested animals (GF-TADs, 2012). Linthicum *et al*. (2016) stated that one of the methods used to protect animals from being infected by RVFV during severe outbreaks is to move them to a higher altitude, far away from the wetlands. However, through advancing technology, scientists have developed a satellite remote sensing technique to monitor and provide early warning of eco-climate changes that can lead to RVF outbreaks. Satellite remote sensing technique has been in existence over the last 30 years (Anyamba *et al*., 2012). In 2006 in East Africa, this system was successfully used to predict early warnings of the RVF outbreaks (Anyamba *et al*., 2009). Satellite remote sensing techniques are used to measure changes in sea surface temperatures that lead to changes in weather and climate affecting the variability in rainfall on an interannual basis. These variations influence the availability of appropriate habitats for RVF vectors. Studies have used normalised difference vegetation index (NDVI) data derived from the Advanced Very High-Resolution Radiometer sensor (AVHRR) instrument on-board the National Oceanic Atmospheric Administration (NOAA) of polar-orbiting satellites (Linthicum *et al*., 1987; Anyamba *et al*., 2001). Anomalies

in NDVI and ecological conditions that are associated with above-normal rainfall, which floods *dambo* habitats, are monitored (Linthicum *et al*., 1987; Anyamba *et al.,* 2001). Also, Williams *et al*. (2016) suggest that rainfall is directly related to soil water saturation and the growth of vegetation. Therefore, measurements of soil saturation index can also be used as an indicator to predict RVF outbreaks as shown in Figure 8. Through remote sensing, vegetation, soil moisture and groundwater level measurements can be measured and can detect when the soil is fully saturated and when wetlands will flood after heavy rainfall (Williams *et al*., 2016).

It is not easy to convince farmers and veterinary specialists to vaccinate susceptible livestock for RVF, mainly because the vaccines are costly. There are other diseases that farmers battle with that occur annually, while large outbreaks of RVF are likely to occur after few years (Linthicum *et al*., 2007; Anyamba *et al*., 2012). Therefore, through the use of remote sensing early warnings systems, RVF outbreaks can be forecasted and predicted at least 2 to 3 months before they occur. This would allow farmers and veterinary authorities to take preventative and control measures in advance, such as animal vaccination to prevent animals contracting the disease and prevent abortion (Linthicum *et al*., 2007; Anyamba *et al*., 2012). The RVF early warning system is necessary because it is not only low cost (Martin *et al*., 2008), but Davies (2010), reported that vaccinating livestock during the epizootic periods can be dangerous and further spread the virus (e.g. the same needles are used to vaccinate the animals). Other preventive measures include mosquito control and public awareness (Linthicum *et al*., 2007; Anyamba *et al*., 2012; GF-TADs, 2012).

In Figure 8, the arrows indicate the first occurrence of the outbreak in each area. The black bars indicate the rainfall, the white bars indicate NDVI, and the grey bars indicate the SSI. Graph A indicates outbreaks in the Southern region of KwaZulu-Natal Province from February to March 2009; B is the outbreaks in the Orange River region in Northern Cape Province in October – November 2009; C outbreaks in Bultfontein area of Free State Province in January – February 2010 and D outbreaks in Graaff-Reinet area of Eastern Cape Province in January – February 2011 (Williams *et al*., 2016).

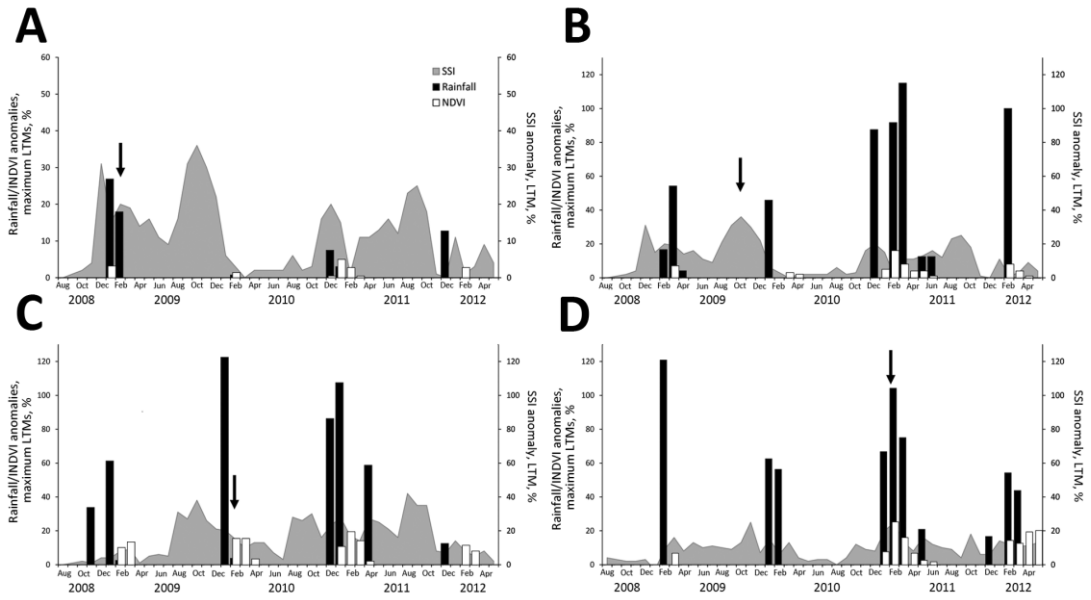


Figure 8 The relationship between rainfall, NDVI and soil saturation indices observed in four different areas within South Africa that were affected by RVF outbreaks between August 2008 and May 2012 (Williams *et al*., 2016).

Climatic conditions, particularly rainfall together with environmental factors such as wetland soils, geology and vegetation, play a considerable role in the occurrence of RVF epidemics (Dautu *et al*., 2012). Furthermore, during periods of above-normal rainfall, cloudy and humid conditions may increase RVF vector populations and improves their survival (Davies *et al*., 1985; Linthicum *et al*., 2016). Floodwater *Aedes* mosquitoes transmit the virus and it is believed that the virus can be transmitted transovarially from female floodwater *Aedes* mosquito to their offspring through their eggs (Davies *et al*., 1985; Martin *et al*., 2008). However, there is still insufficient research on transovarial transmission and how the environmental factors may affect the survival of *Aedes* mosquito eggs in wetland soil for such a long time (Logan & Linthicum, 1991). It has been suggested that all environmental variables that favour the occurrence and growth of floodwater *Aedes* mosquitoes should be monitored (Linthicum *et al*., 2016), this includes soil properties.

2.4.7 Life cycle of the Rift Valley fever virus

Past research indicates that female floodwater *Aedes* mosquitoes lay infected eggs on the edges of desiccated wetlands (also known as *dambos*/*pans/vleis*) in grassland biomes where they can survive through successive dry years, over long periods and Davies *et al*. (1985) suggest it takes 5 to 15 years. The entire cycle depends on above-normal rainfalls, flooding of *dambos,* and transovarial transmission. Immediately after heavy rainfall, the water table rises, which leads to flooding of these poorly drained low-lying habitats. After a day or two of flooding, the eggs that have been inactive on the soil will start to hatch and produce large numbers of *Aedes* mosquitoes, some of which are infected by the virus (Linthicum *et al*., 2016). Vegetation

within the wetland will act as a habitat for the immature *Aedes* mosquitoes until the adult stage. These infected *Aedes* feed on ruminants in wetlands. The infection causes a high viraemia and thereby initiates a new epizootic cycle (Aradaib, 2010; Linthicum *et al*., 2016; Figure 9). Vectors such as *Culex* and *Anopheles* feed on the infected animals, contract the virus and then transmit it further to ruminants and humans (Linthicum *et al.*, 1999; FAO/WHO 2008; Linthicum *et al*., 2016).

The movement of animals during an outbreak is a risk factor because it allows the epizootic cycle to start in a new place and spread the disease (FAO/WHO 2008). Furthermore, irrigation schemes and newly constructed dams may result in flooding of river banks and thus lead to a rise of the water table, flood wetlands and eggs hatch and thus cause RVF outbreaks (Balkhy & Memish, 2003; Williams *et al*., 2016). One example is in Egypt between 1977 and 1979, where a massive epidemic occurred which was related to irrigation (Linthicum *et al*., 2016). This also occurred in the Senegal River basin, which includes northern Senegal and southern Mauritania. The outbreaks were associated with newly constructed dams on the Senegal River (GF-TADs, 2012). The Northern Cape Province in South Africa experienced an outbreak associated with irrigation schemes (Thompson & Pienaar, 2013).

The diagram on the right in Figure 9 represents epidemics and epizootics of RVF where prolonged heavy rainfalls flood the *pans* where floodwater *Aedes spp.* mosquito eggs were dormant for several years. Upon flooding, within a day or two, dormant infected eggs hatch into feeding *Aedes* mosquitoes that reintroduce the RVFV locally. Surrounding domestic ruminants are the first to be bitten by these newly hatched mosquitoes which also serve as the amplification hosts for RVFV. Secondary vectors such as *Culex spp* obtain the virus through a blood meal of infected animals, thus contributing to the transmission of RVFV to other animals and humans (Linthicum *et al*., 2016).

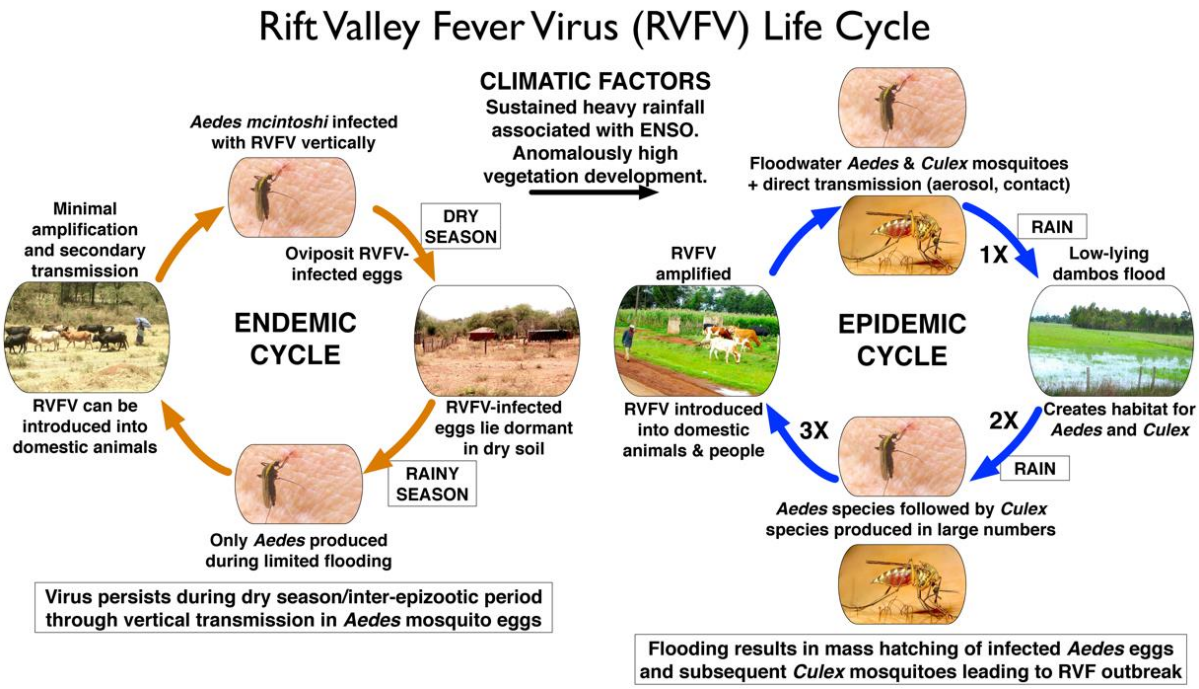


Figure 9 An endemic cycle where temporal flooding of *pans* result in infected *Aedes* mosquitoes (left) and thus transmit the virus to the vertebrate host under suitable conditions (right; Linthicum *et al*., 2016).

Chapter 3: Description of the study area

3.1 Location

The study area consisted of approximately 200 km by 200 km of the Free State and Northern Cape Provinces in South Africa as shown in Figure 10 and Table 6. These two provinces were selected for this study because most of the outbreaks occurred there in 2010/2011. Figure 10 lists the 30 quaternary research stations and indicate their unique farm identity (ID), the nearest town, coordinates, whether the sites reported RVF mortalities and HGM units. Each of these sites differs in terms of climate, hydrology, lithology, soils and vegetation.

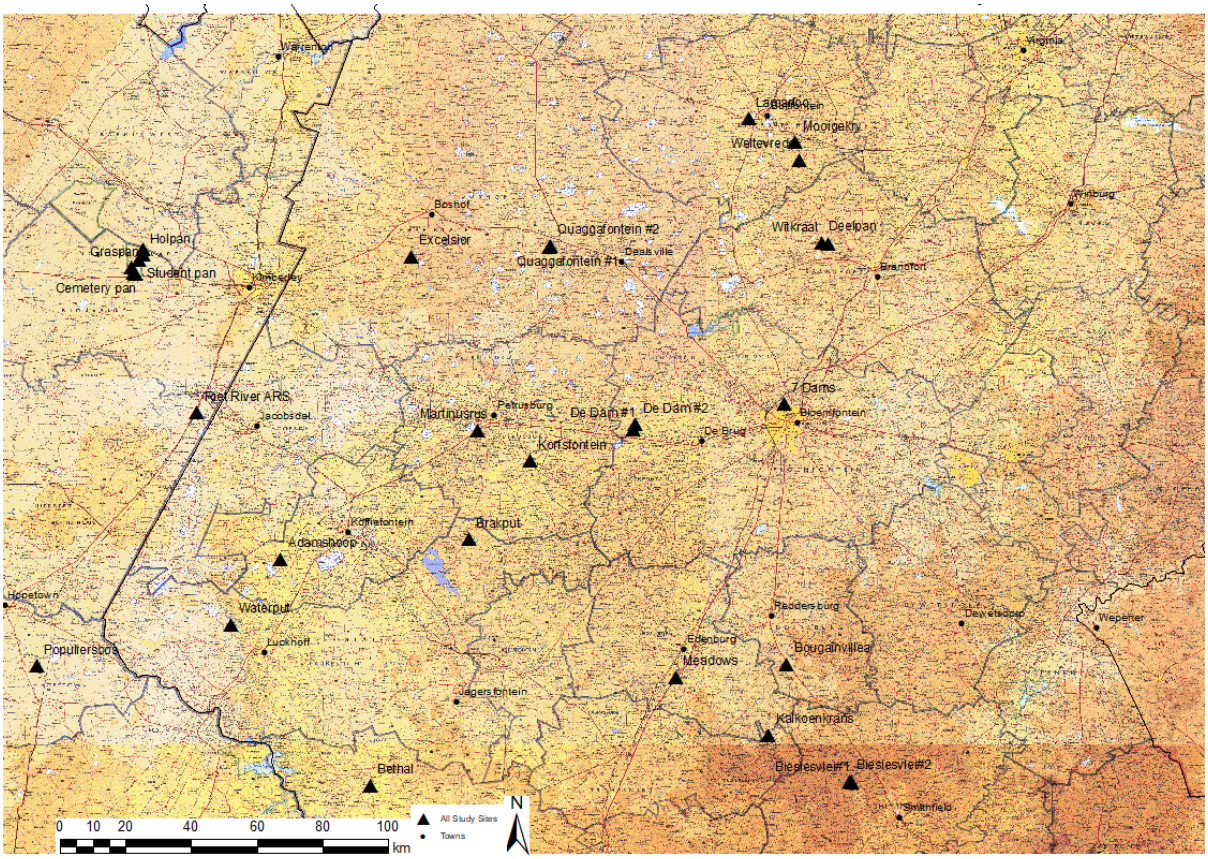


Figure 10 The study area, including the location of the study sites.

Table 6 Farm ID, the nearest town, GPS coordinates, reported RVF mortalities and HGM

Farm ID	Town	Longitude (South)	Latitude (East)	Mortalities	Hydrogeomorphic unit
p013b	Brandfort	28,63	26,29	Reported (Outbreak)	Depression (includes *pans*)
p001b	Brandfort	28,37	26,85	Reported (Outbreak)	Depression (includes *pans*)
p002b	Bultfontein	28,40	26,25	Reported (Outbreak)	Depression (includes *pans*)
p004b	Bultfontein	28,28	26,11	Reported (Outbreak)	Valley-bottom unchannelled

Farm ID	Town	Longitude (South)	Latitude (East)	Mortalities	Hydrogeomorphic unit
p009d	Dealsville	28,63	25,57	Reported (Outbreak)	Valley-bottom unchannelled
p009d	Dealsville	28,63	25,57	Reported (Outbreak)	Depression (includes *pans*)
p011p	Petrusburg	29,13	25,37	Reported (Outbreak)	Depression (includes *pans*)
p010j	Jacobsdal	29,09	24,60	Reported (Outbreak)	Wetland flat
p008o	Koffiefontein	29,49	24,83	Reported (Outbreak)	Valley-bottom unchannelled
p007l	Luckhoff	29,40	24,42	Reported (Outbreak)	Anthropogenic
p005p	Koffiefontein	29,43	25,35	Reported (Outbreak)	Depression (includes *pans*)
p012r	Reddersburg	29,46	26,20	Reported (Outbreak)	River
p014b	Bloemfontein	29,06	26,21	Reported (Outbreak)	Valley-bottom channelled
p003b	Bultfontein	28,35	26,24	Not reported (No outbreak)	River
p006b	De Brug	29,12	25,80	Not reported (No outbreak)	Depression (includes pans)
p006b	De Brug	29,13	25,80	Not reported (No outbreak)	Valley-bottom channelled
p015k	Kimberley	28,41	24,25	Not reported (No outbreak)	Depression (includes pans)
p015k	Kimberley	28,64	24,45	Not reported (No outbreak)	Depression (includes *pans*)
p015k	Kimberley	28,40	24,26	Not reported (No outbreak)	Depression (includes *pans*)
p015k	Kimberley	28,70	24,43	Not reported (No outbreak)	Depression (includes *pans*)
p015k	Kimberley	28,71	24,26	Not reported (No outbreak)	Depression (includes *pans*)
p015k	Kimberley	28,71	24,44	Not reported (No outbreak)	Depression (includes *pans*)
321h	Hopetown	29.78	24.16	Not reported (No outbreak)	Valley-bottom channelled
222p	Phillipolis	30,11	25,12	Not reported	Valley-bottom channelled
259e	Edenburg	29,78	25,94	Not reported (No outbreak)	Valley-bottom channelled
211b	Boshof	28.66	25.19	Not reported (No outbreak)	Valley-bottom channelled
284p	Petrusburg	29,23	25,51	Not reported (No outbreak)	Valley-bottom channelled
131s	Smithfiled	30,12	26,41	Not reported (No outbreak)	Valley-bottom channelled
131s	Smithfiled	30,10	26,39	Not reported (No outbreak)	Valley-bottom channelled
140t	Reddersburg	29,96	26,14	Not reported (No outbreak)	Valley-bottom channelled

3.2 Climate

South Africa is characterised by a wide variety of climate due of different climatic zones .The climatic zones further influence the nine biomes of South Africa. Areas that receive less than 600 mm of rainfall fall under dry Highveld grassland and this is where most of the study sites are located. In these regions, rainfall and temperature are erratic with minimal rainfall and extended drought periods. For example, the study areas that are located in Luckoff, Edenburg, and Smithfield are categorised as Karroid Grassland, these receive mean annual precipitation (MAP) of (490 mm) and have a mean annual temperature (MAT) of about 15ºC. Sites in Bloemfontein, Petrusburg, Boshof and Brandfort fall under dry grassland, with a (MAP) of 450 mm and a MAT of about 16°C Figure 11. These regions are characterised by late summer rainfall while frost is common (Lotter *et al*., 2006). Study areas situated at Kimberley in the Northern Cape are part of the Savanna biome and are characterised by rainfall gradient (300 mm in the southwest to 500 mm in the northeast). The temperatures in the study sites within the Northern Cape range from 37.5ºC in January to 4.1ºC in July (Rutherford *et al*., 2006). Hopetown is situated in Nama Karoo (Rutherford *et al*., 2006).

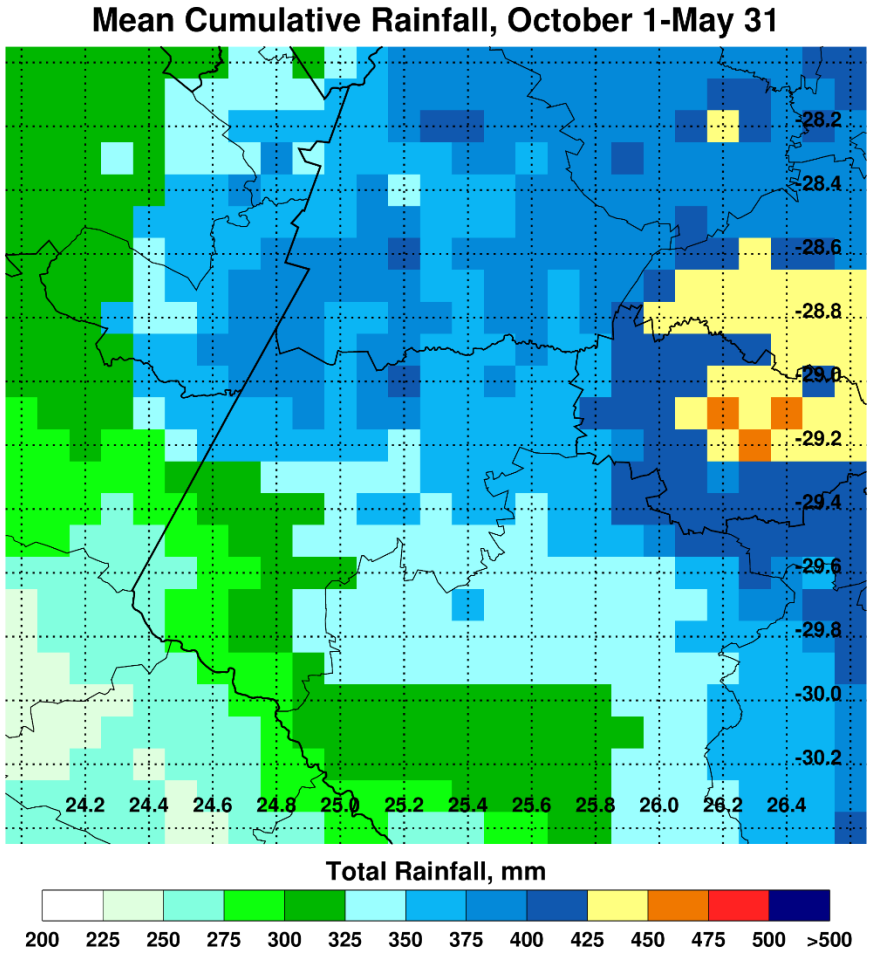


Figure 11 Annual rainfall received for the study sites from 1983 to 2017 (Anyamba, 2018. Personal communication).

3.3 Geology

Shale and sandstone of the Karoo Super Group rocks mainly dominate the geology of the Free State and Northern Cape Provinces. According to Johnson *et al*. (2006), these rocks were deposited about 300 million years ago during the Late Carboniferous and middle Jurassic period. Their deposition occurred in distinct environments such as glacial, deep marine, shallow marine, deltaic, fluvial, lacustrine and aeolian (Johnson *et al.,* 2006). Sedimentary rocks of the Karoo Super Group dominates the geology of the region. In South Africa, this group underlies approximately two-thirds of the land area (Johnson *et al.,* 2006). The Karoo Super Group is subdivided into five main groups, namely the Dwyka, Ecca, Beaufort, Stormberg and Drakensberg Groups. All these groups consist of sedimentary rocks, whereas, the Drakensberg Group consists of volcanic rocks (Johnson *et al.,* 2006). Only Dwyka, Ecca and Beaufort will be discussed further since they are within the boundaries of the study area. However, it is important to highlight that Dolerite intrusions are common throughout the sampling area.

Study sites around Bultfontein, Dealsville, Petrusburg, Koffiefontein, Luckhoff, Boshof and Hopetown are mainly dominated by the Ecca group, whereas the study sites around Bloemfontein, Brandfort, Phillipolis, Jacobsdal, Edenburg, some parts of Reddersburg and Smithfiled are dominated by Beaufort group. The Dwyka group dominates study sites around Kimberley (Figure 12)

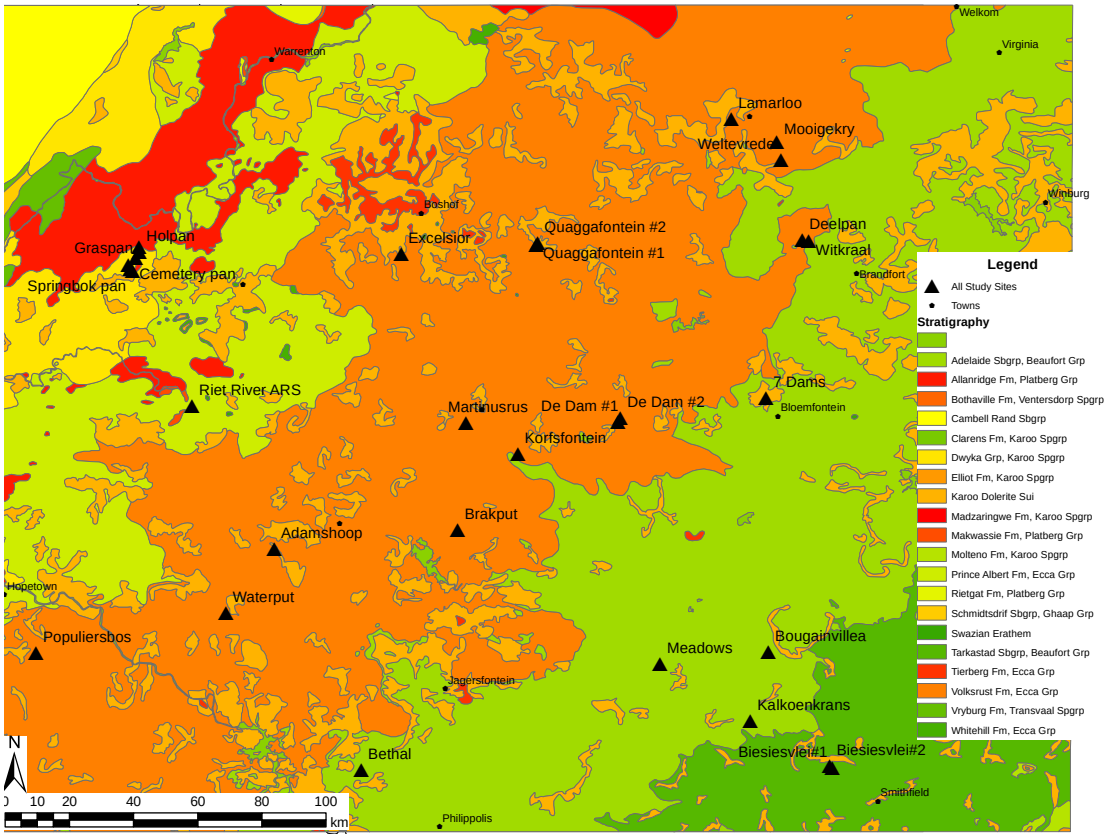


Figure 12 Distribution of geological groups across the study area.

3.4 Karoo Super Group

3.4.1 Beaufort Group

The Beaufort Group dominates most of the Free State. This group overlies the Ecca Group and is further subdivided into Tarkastad and Adelaide groups. This group is primarily dominated by sedimentary rocks that are rich in fossiliferous sandstone and mudstone (Johnson *et al.,* 2006). The Eastern and Central Free State is well represented by this group (Holmes & Barker, 2006). Study sites in Bloemfontein, Reddursburg and Edenburg are dominated by mudstone and sandstone of the Adelaide subgroup (Lotter *et al*., 2006).

3.4.2 Ecca Group

The Ecca Group is enclosed between the Dwyka and Beaufort Groups. This group is characterised by dark grey shale, sandstone, siltstone, mudstone, conglomerate and coal (Johnson *et al*., 2006). The Western Free State is well dominated by this group and study sites in Bultfontein and Boshof fall into this group (Lotter *et al*., 2006; Holmes & Barker, 2006).

3.4.3 Dwyka Group

The Dwyka Group was deposited during the Late Palaeozoic glacial episode and is composed of the oldest rocks (Isbell *et al*., 2008). This group is the foundation of the Karoo Super Group in South Africa and consists of shale, tillite, conglomerate and sandstone (Johnson *et al*.,

2006). Furthermore, the Dwyka Group occurs in some parts of the Western Free State as well as in some parts of the Northern Cape (Holmes & Barker, 2006).

3.5 Topography

The topography of the Free State is fairly flat with 3291 m.a.s.l. Similarly, the Western part of the province is generally flat too, with irregular plains and is dominated by a high number of pans, sites that include (211bosxcls, p009deaqwgg #1 & 2, p008oppdmsh, p005petbrkp, p007lucetrp, p013bradlpn, p001brawtkr, p002bulwltv p004bullmrl, p011petmrtn, p006bftddmm #1 & 2, 284petkrfs, p003bulmgkr & p010jacrtrv) are situated in the western Free state. Goudie and Wells (1995), reported that the Western Free State has the highest *pans* in the world i.e. (1200 m.a.s.l). In contrast, the central (p014blo7dms) and southern parts (222phibthl, 140troklkn, 284petkrfs, 131smibssv#1 & 2 , 259edemdws &p012redbgnv) of the province are dominated by lowlands with hills, while the eastern parts of the province are characterised by mountainous areas with irregular plains and hills i.e. (1800 m.a.s.l) (Holmes & Barker, 2006). The Northern Cape (321hoppplr & p015kimgrsp) is characterised by flat landscapes (less topographical relief) with isolated hills and mountains.

3.6 Soil

Soil is formed through five factors which include parent material, topography, climate and biota that all interact with each other over time (Jenny, 1994; Hensley *et al*., 2006). According to Hensley *et al*. (2006), out of all the five forming factors, parent material and climate played a leading role in the soil formation of the Free State. Areas of Philipollis and Smithfiled are dominated by duplex soils. Study areas located in Petrusburg, Koffifontein, parts of Bulfontein and Boshof are dominated by red, yellow, structureless freely drained soils. Bloemfontein and Brandfort sites are dominated by dark clay soils with swelling and shrinking properties as well as duplex soils with an intrusion of dark coloured margalitic clay soils that have swelling and shrinking properties. All study sites in the Free State are situated in the arid and semi-arid regions, with rainfall received equal to or less than 500mm. Areas such as Philipollis, parts of Koffifontein, Boshof, Edenburg, and Reddusrburg are dominated by non-arable soils. Bloemfontein, Petrusburg, Brandfort, and some parts of Smithfield are characterised by the soils with very low potential. The Bulfontein area is dominated by low potential soils. The Northern Cape soils are often dominated by a reddish colour, sandy features, moderately shallow, a weak structure, and are well-drained with low organic content (Rutherford *et al*., 2006).

3.7 Vegetation

South Africa is characterised by a wide range of biomes described by Mucina and Rutherford (2006) as highlighted in Figure 13. The Grassland biome is the second-largest biome (27.9%) after the Savanna biome (32.5%) (Rutherford *et al*., 2006). The Grassland biome dominates a large area of central South Africa and is further subdivided into bioregions based on the annual rainfall received (Lotter *et al*., 2006). Most study sites are situated in the grassland biome under dry Highveld grassland bioregion. The grass type that is predominant in this region is sweet grassland, which belongs to the *Chloridoid* subfamily. Within this family, the soils are less leached and there is also lower plant production and fire frequency. The following grasses dominate areas in Edenburg, Smithfield, and Luckhoff: *Themeda triandra, Cymbopogon persichilli*, and *Digitaria eriantha*. Shrubs are also common. The *Themeda triandra grass* also dominates areas in Bloemfontein, Petrusburg, and Reddersburg (Lotter *et al*., 2006). Brand *et al*. (2018) conducted a vegetation study based on 15 out of the 22 wetlands. The study was conducted by surveying 129 transects, of which 158 plant species were identified. These included sedges, grasses, rushes, bulrushes, and sub-shrubs. All sites with reported RVF mortalities were dominated by sedges and juncus and they are obligate and facultative wetland species. Furthermore, several RVF mortalities were compiled for different plant communities and dominant vegetation associated with RVF mortalities. Plant species were further classified into eight different communities as shown in Table 7. The study provided wetland characteristics from areas where known RVFV mortalities occurred.

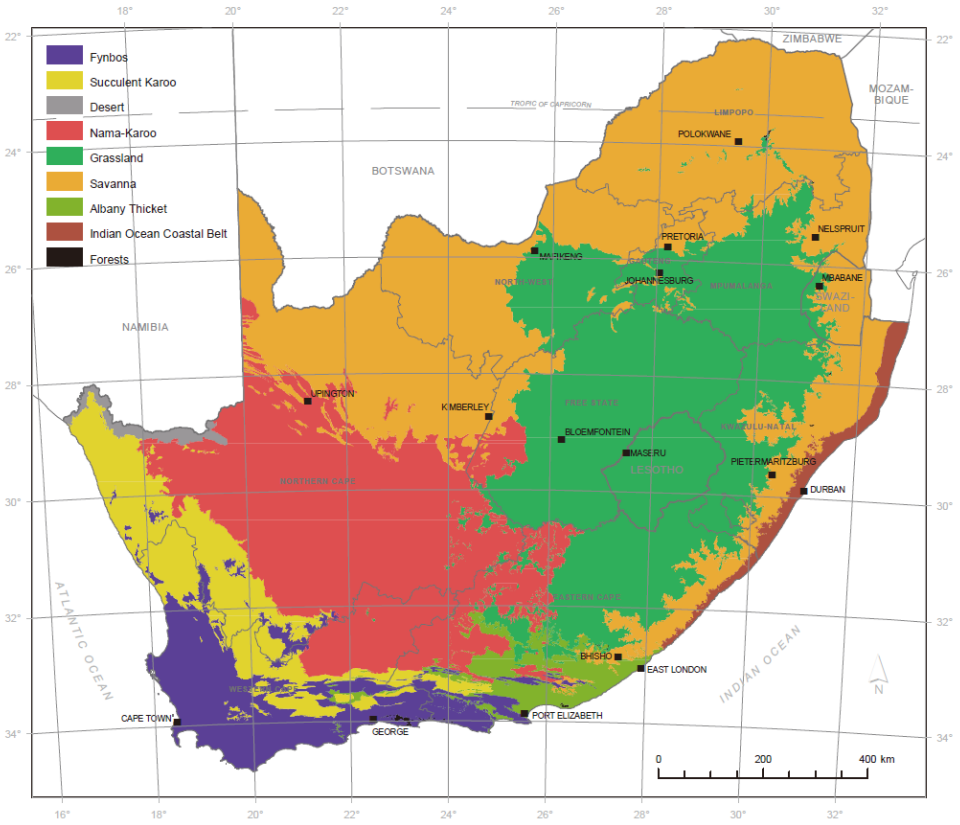


Figure 13 Different biomes in South Africa. The study sites in the Free State are dominated by Grassland biome, the Northern Cape is dominated by the Savanna and Nama Karoo biome (Rutherford *et al*., 2006).

Table 7 Vegetation communities with grass species associated with RVF mortalities (Adapted from Brand *et al*., 2008)

Community	Grass species	RVF mortalities (2010/2011)
Community 1	*Eragrostis bicolor*, and *Eragrostis obtusa*	Not reported
Community 2	*Cyperus laevigatus* and *Agrostis lachnanthes*	Reported
Community 3	*Fuirena coerulescens* and *Echinochloa colona*	Reported
Community 4	*Hemarthria altissima* and*Schoenoplectus muricinux*	Reported
Community 5	*Cyperus laevigatus, Pseudschoenus inanis*	Reported
Community 6	*Agrostis lachnantha, Cyperus longus*	Not reported
Community 7	*Scirpoides dioecious and Juncus rigidus*	Reported
Community 8	*Cyperus laevigatus and*Juncus *rigidus*	Reported

Chapter 4: Material and methods

4.1 Site selection

This study continues the work of Verster (2016), where previously the study was conducted on twenty-two (22) sites, selected based on the suitability of breeding sites for mosquitoes (i.e. vegetation, wetland type, size, and soil). For all these sites, the farmers'/landowners' permission was granted. Of the 22 sites, 12 were selected based on locations that reported mortalities during the 2010 and 2011 outbreak, while ten sites did not report livestock mortalities (Verster, 2016). The study was subsequently expanded to include eight additional sites that were selected based on the cross-sectional study of the farms that had seropositive sheep and were never vaccinated. For the privacy of the farmers, farm IDs were used in this dissertation.

All the 30 study sites were classified using a 5 km buffer and all the sites falling inside this area were classified as mortality sites, while the rest were classified as sites without mortalities. It is important to note that previously, p014blo7dms was considered as a site without RVF mortalities, however, it was reclassified as a site with RVF linked mortalities because it was too close to the buffer zone.

In November 2016, a multi-disciplinary team of scientists with different environmental specialities (soil scientists, entomologists, veterinarians, climate, and vegetation specialists) visited the eight additional farms to select wetland sites. The criteria for the wetland site selection were as follows: 1) sheep utilising the wetland, 2) type of wetland (HGM unit), 3) size of the wetland, 4) dominant wetland vegetation, and 5) soils that indicate signs of wetness. The sites were revisited during February and March 2017 to collect soil samples, conduct soil classification, and several installations. These installations included Davis weather stations, level loggers, and soil water sensors/probes (watermark and ECH2O 5TE sensors). Table 8 gives a monitoring inventory including the Farm ID with the different numbers of level loggers and water probes that were installed per site.

Table 8 Farm ID, level logger number, soil moisture probes, Barro loggers and RVF reported mortalities

Farm ID	Level logger No.	Soil Moisture probes	Barro loggers	Mortalities
p013bradlpn	102043931	Watermark sensors	12043540	Reported
p001brawtkr	102043932	EM 32730	12043540	Reported
p002bulwltv	102044408	EM 32723	12043540	Reported
p004bullmrl	102044433	EM 32724	12043540	Reported
p009deaqwgg	102044442	Not installed	12043815	Reported
p009deaqwgg	102044448	Watermark sensors	12043815	Reported
p011petmrtn	102069154	EM 32725	12045560	Reported
p010jacrtrv	102045247	EM 32729	12045560	Reported
p008oppdmsh	102045249	Watermark sensors	12045560	Reported
p007lucetrp	102045251	EM 32722	12045560	Reported
p005petbrkp	102070761	No installed	12045560	Reported
p012redbgnv	102044452	EM 32720	12043836	Reported
p003bulmgkr	102044432	EM 32728	12043540	Not reported
p014blo7dms	102043930	EM 32727	12043531	Not reported
p006bftddmm	102044454	EM 32721	12043836	Not reported
p006bftddmm	102044456	Not installed	12043836	Not reported
p015kimgrsp	102045243	Not installed	12045058	Not reported
p015kimgrsp	102045197	Not installed	12045058	Not reported
p015kimgrsp	102045219	Not installed	12045058	Not reported
p015kimgrsp	102045232	Not installed	12045058	Not reported
p015kimgrsp	102045239	Not installed	12045058	Not reported
p015kimgrsp	102045242	Not installed	12045058	Not reported
321hoppplr	102070760	Watermark sensors		Not reported
222phibthl	102069146	Watermark sensors		Not reported
259edemdws	102070868	Watermark sensors		Not reported
211bosxcls	102070871	Watermark sensors		Not reported
284petkrfs	102070759	Watermark sensors		Not reported
131smibssv	102070860	Watermark sensors		Not reported
131smibssv	102069139	Watermark sensors		Not reported
140troklkn	102070762	Watermark sensors		Not reported

4.2 Soil samples

Thompson and Edelman soil augers were used to sample the soil. Soil was sampled at the edges of wetlands in the same location. Soil samples consisting of six subsamples were collected at each diagnostic horizon. In some sites where the bedrock was shallow, for example, at 211bosxcls, the soil was only sampled to a depth of 700 mm. Hydrochloric acid (10% v/v) was used to test the presence of lime in the soil. Soils were described (Turner, 1991) and classified according to the Soil Classification Working Group guidelines (1991). The classification was further done according to the standards of WRB IUSS Working Group (2006). Soil samples were stored in plastic bags, labelled, and transported to the laboratory where they were oven-dried at 60°C for more than 24 hours. The soil was crushed, sieved through a 2 mm sieve, and stored in 1 L polyvinyl chloride (PVC) containers for laboratory analyses.

4.3 Laboratory analyses

Non-Affiliated Soil Analysis Work Committee (1990) standard analytical procedures were used for most chemical and physical analyses. All analyses were done in replicates of two for each horizon (topsoil and from each master horizon). The analytical results available from the previous study conduct by Verster (2016) were used in this study. However, additional analyses that included Olsen, water, and calcium chloride P extractions as well as electrical conductivity, were compiled and completed as needed.

Physical properties were assessed based on texture using a pipette method. This method was characterised as one of seven fractions: sand (2.00-0.05 mm; coarse, medium, fine and very fine sand), silt (0.05-0.02; coarse and fine silt), and clay fractions (<0.002 mm). Before texture analysis, the topsoil was treated with acidified NaOAc to remove carbonates and with H_2O_2 to remove organic matter.

Chemical analyses comprised of pH (KCl and H_2O), electrical conductivity, electrical resistance (soil paste), soluble and exchangeable cations, CEC (1 mol dm^{-3} NH_4OAc at pH 7.0), organic carbon (Walkley-Black), nitrogen (Kjeldahl) and phosphorus. Phosphorous was analysed using the Olsen method (Non-Affiliated Soil Analysis Working Committee, 1990), and by the water and calcium chloride ($CaCl_2$) extractions (Pierzynski, 2000).

4.3.1 Mineralogical analyses

Mineralogical analyses consisted of fluorescence (XRF) and X-ray diffraction (XRD) analyses. Both XRF and XRD were performed by the Department of Geology at the University of the Free State. The analysis of mineralogical analyses were conducted only for the top soil. XRF is an analytical technique used to identify major and trace elements (Potts, 2004). These analyses were conducted to determine the elements that could potentially best relate to the occurrence of RVFV.

Ten gram of topsoil (0-50mm) was heated to 110°C for 24 hours to dehydrate and devolatilize the sample. Immediately after that, the temperature was increased to 1050°C to further break down minerals such as carbonates. The mixture (0.2445 g of La_2O_3, 0.705 g of $Li_2B_4O_7$, 0.5505 g of Li_2CO_3 and 0.02 g of $NaNO_3$), was then added to 0.28 g of the sample. The whole mixture was thereafter heated at a temperature of 1000°C for roughly 5 minutes until a steady fluid was formed within a Platinum (Pt) crucible. The fluid was then poured into a mould and pressed to form a disc. Standards were prepared to measure the major elements and the machines were conditioned for each element measured. Additional standards were also prepared to measure the trace elements (Willis & Duncan, 2008).

XRD is a technique used to perform structural and crystallographic characterisation of polycrystalline mixtures, identification, and quantification of mineral phases as well as

microstructure and texture analysis (Schulze, 2002; Bortolotti *et al*., 2017). The clay fractions collected during the texture analyses were used for the XRD analysis. XRD was performed using a Panalytical Empyrean X-ray Diffractometer (Malvern Panalytical Instruments Ltd, UK), which is equipped with a copper (Cu) side window tube, an anode containing Cu and a W cathode with an X Celerator detector. The X-ray tube produces a $CuK_{\alpha 1}$ X-ray beam with a Bragg-Brentano geometry focus position along with a Theta-Theta goniometer configuration (Anonymous, 2014).

4.3.2 Total microbial activity

Fluorescein Diacetate Hydrolysis (FDA) was used to determine the total microbial activity in the soil. It estimates the amounts of active fungi, bacteria, and locates acetyl esterases in living protist cells (Schnürer & Rosswall, 1982). FDA hydrolysis is commonly accepted as an accurate and simple method for measuring total microbial activity in a range of soils.

4.3.2.1 Soil sampling, preparation, and storage

Samples for FDA analysis were collected in 2015 between May and June (first sampling period) and again during May 2018 (second sampling period). Samples were taken with a 5 cm diameter Thompson soil auger at a 0-5 cm depth. Six samples were randomly collected from each wetland site and then combined into one composite sample for each site. Microbial samples were hand crushed and sieved through a 2 mm sieve in the field, mixed thoroughly, immediately transferred into plastic bags, labelled and stored on ice or in a refrigerator (approximately 4°C) until analysis. Five replicates were tested from each of the 30 wetland sites.

4.3.2.2 Microbial biomass

The measurement of total microbial activity using the FDA method was conducted by the rapid, yet sensitive method described by Schnürer & Rosswall (1982), as modified by Adam & Duncan (2001). Two (2) g of the moist composite soil sample was weighed and placed into a 50 ml falcon tube. The samples were mixed with 15 ml of 60 mM potassium phosphate (K_3PO_4), buffered at pH 7.6, and 0.2 ml of 1000 µg/ml FDA stock solution was added. Blanks were prepared without the additions of the FDA substrate. The flasks were closed with stoppers, shaken slowly by hand, and then placed in an orbital incubator (100 rpm) at 30°C for 20 minutes. After removing the samples from the incubator, 15 ml of chloroform/methanol (2:1) was added immediately to terminate the reaction. The contents were then transferred to 50 ml centrifuge tubes and centrifuged at 3000 rpm for approximately three minutes to settle the soil. The absorbance was determined at 490 nm wavelength on a spectrophotometer.

4.4 Soil water content

4.4.1 Watermark sensors

Watermark water (Irrometer Soil Water Management since 1951, USA) and temperature sensors were used to estimate water potential between 0 and 200 kPa. A reading between 0 and 10 Cb indicated that the sensor was wet, whereas a dry sensor would read between 100 and 200 Cb (Watermark manual, 2001). The sensors were installed within 10 and 30 m of the Davis weather station (Davis Instruments, USA). Both watermark and temperature sensors were installed in all eight additional sites and a few of the old sites, these were connected to the Davis weather station (Figure 14). Before installation, sensors were soaked in water, allowed to dry, and then get soaked again. This process was carried out to remove air within the sensors and to improve their response (Wireless soil moisture/ temperature station installation manual, 2001). During installation, an auger was used to excavate four holes to depths of 50 mm, 100 mm, 350 mm and 550 mm. The sensor wires that protruded from the soil were labelled according to their respective depths and connected to a data logger that was buried in the hole. The data logger uploaded data hourly to a website (vitalweather.co.za) via the weather station, from where it could be downloaded.

4.4.2 ECH2O 5TE sensors

The ECH_2O 5TE sensors (Decagon devices, Washington) transmit data to an electromagnetic (EM 50) data logger, which is a 5 channel, self-contained data recorder. ECH_2O 5TE is designed to measure volumetric water content (VWC), electrical conductivity (EC), and temperature (Cobos & Chambers, 2010). These sensors were installed at sites that did not have a Davis weather station (Figure 14). Installation required a hole with an area of 50 cm^2. The four moisture sensors were installed horizontally at the same depth as the watermark sensors, to ensure consistency. The protruding sensor wires were carefully labelled according to their depths and inserted into 4 channels of the EM 50. The data from the sensors was recorded hourly and collected monthly using a logger reader and the ECH_2O utility software.

4.4.3 Level loggers

Solinst (model 3001) (Solinst, Canada) level loggers were installed in all 30 sites (Figure 14) to measure ground and surface water levels and temperature (Level Logger User Series Manual Guide, 2018). The UFS Instrumentation Department drilled (perforated) 50 mm diameter PVC pipes to construct wells for the installation of level loggers. A 1200 mm hole was excavated using an Edelman auger into which the well-pipes, were inserted at each site. The Solinist level loggers were then initialised, tied to a string, and placed into the well to monitor water levels at hourly intervals. The data from the level loggers was collected with a level loader/data grabber and downloaded using the latest Solinst software (version 4.40).

Barro loggers were installed at the sites (Figure 14) and were used as a reference to calculate the water levels, using the level logger software (version 4.4.0).

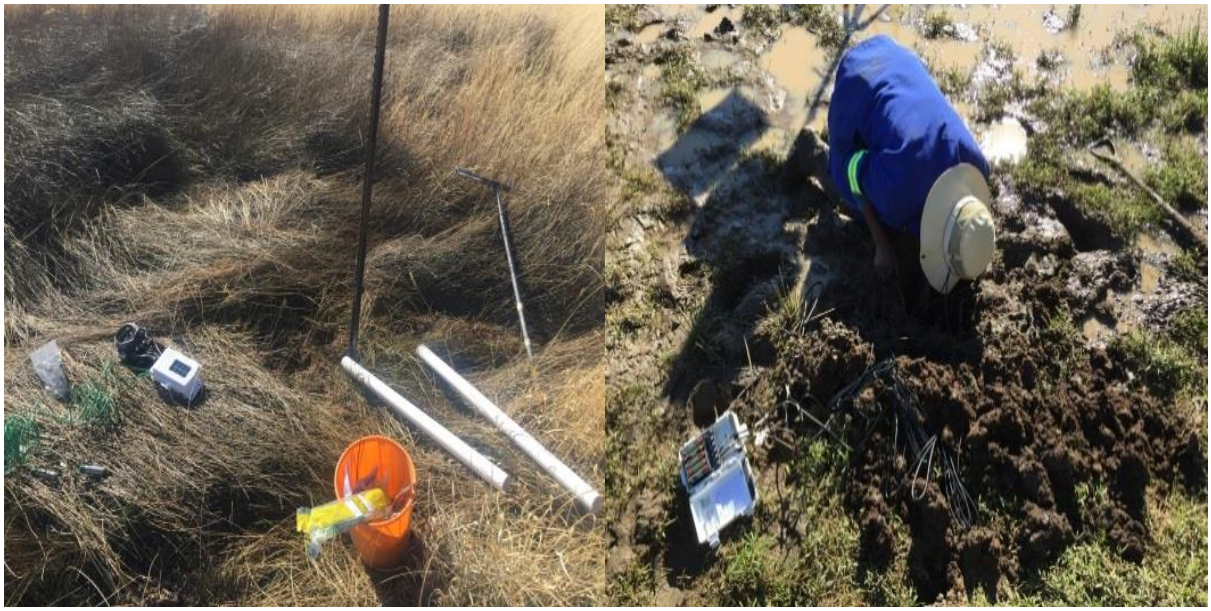


Figure 14 Installation of the Watermark moisture sensor (left), the ECH2O 5TE sensors (right).

4.5 Sensor calibration

ECH2O probes measure the volumetric water content (VWC) of the soil by measuring the dielectric constant of the soil, which is a strong function of water content (Cobos & Chambers, 2010). The commonly used calibration procedure to quantify soil water sensors is the gravimetric water content (GWC). The advantage of the GWC method is accuracy, alternatively, the disadvantage is that it is labour intensive and time-consuming. Calibration can be done either in the field or in the laboratory (Parvin & Degré, 2016; Starr & Paltineanu, 2002). For this study, the following field procedure was followed to calibrate the water content:

- All study sites with water probes were visited (Figure 14) to collect the bulk density of the soil to determine GWC and VWC water content.
- Four (4) different undisturbed core samples were collected at each horizon using a PCV ring that was fixed into a metallic core sampler and driven vertically into the soil.
- The two (2) ends of the core sampler were neatly trimmed and transferred into brown paper bags.
- The paper bags were sealed to prevent water loss.
- The samples were taken to the laboratory.
- A wet mass was weighed immediately and oven-dried at 105 ˚C for over 24 hours to ensure that the samples are completely dried.

- The mass of oven-dried mass was weighed. The mass of the oven dry sample was obtained after 24 hours.

The same procedure was repeated three times at different water contents (dry soil, fully saturated soil, and partially saturated soil). Following this field procedure was necessary to obtain the calibration curve (Cobos & Chambers, 2010).

4.6 Statistical analyses

Statistical analyses were performed using SAS Institute Inc. (2013). Three analyses were performed: for the descriptive analysis the mean, standard deviation, minimum, median, and maximum were calculated by layer if applicable (some sites had only three layers whereas others had four layers) for all sites and by batch (previously sampled versus new sampled). The second analysis performed was between-group comparisons. For this, various soil variables were compared between groups using the non-parametric van der Waerden test. Where applicable, the comparison was carried out in layers, i.e. layers one-to-four. For the averages of the data only layers, one to three were analysed because the fourth layer was not collected from all sites. For each test, the p-value was reported, testing the null-hypothesis of no difference between the groups. Lastly, using a discriminant analysis, the new sites were classified using the discriminant function that was developed using the data from a previous analysis (Verster, 2016) to validate the existing discriminant function.

4.7 Discriminant function

Discriminant function analysis is a multivariate statistical analysis of variance that predicts the group from a set of predictor variables (Poulsen & French, 2008). It is used to determine the quantitative variables or predictors that best discriminate between two groups or more (Ramayah *et al*., 2010). It can also be used to build a predictive model of group membership based on observed characteristics of each case (Oyedeji, 2015).

Chapter 5: Wetland soil properties and RVF vectors

5.1 Introduction

A variety of viruses, that have the potential to cause infectious diseases, exist in the soil environment. These viruses pose serious health risks to humans, animals, plants, and can also infect insects and microorganisms (Ferra & Bilton, 1990). Climate variations and weather conditions play an important role in these infectious diseases and amongst all these variables precipitation, temperature, radiation, and wind all potentially affect their reproduction, survival, and distribution (Wu *et al*., 2014; Wu *et al*., 2016). Hoshen & Morse (2004) indicate that most vector-borne diseases, including RVFV, are mostly influenced by rainfall and temperature. RVF is influenced by other environmental factors such as geology, geography, and soil properties (Njenga & Bett, 2019; Hightower *et al.*, 2012). There is a great variety of soil properties that have previously been reported to play a significant role in the survival and movement of viruses within the soil. These include soil pH, soil texture, soil type, clay minerals, salinity, organic matter, heavy metals, temperature, moisture content, etc. (Kimura *et al*., 2007). All these properties are interdependent they influence each other. Although the RVFV is not directly located in the soil, it is within the mosquito eggs, which can remain dormant in the soil for a certain period. The same soil properties mentioned above are still being investigated in this study to find out how they might play a role in virus activation. This chapter addresses the first objective of this study: to evaluate wetland soil properties that best relate to the occurrence and absence of RVF vectors, based on the sites with RVF mortalities and sites without RVF mortalities classification.

5.2 Chemical properties

Detailed summary statistics and analytical data are presented in Appendix B.

5.2.1 Soil organic carbon

At all sites, the organic carbon content varied widely across the soil profile (mean = 3.38 to 1.57%). The topsoil always had the highest levels of organic carbon (mean = 3.38 and 2.98%, for sites with and without mortalities respectively), but there was no noticeable trend as the depth increased (Table 9). The sites with RVF mortalities had the highest organic carbon over all depths (mean = 2.66%) when compared to the sites without mortalities (mean = 2.14%), but the difference was not significant (Table 10).

5.2.2 Total nitrogen

At all sites the total nitrogen showed a variation in all soil depth (mean = 1.67 to 6.93%; Table 9). There was no significant difference observed between sites with RVF mortalities and sites without mortalities Table 10.

5.2.3 Phosphorus

Three different methods were used to analyse phosphorus in this study, including calcium chloride ($CaCl_2$), water (H_2O), and Olsen extraction. $CaCl_2$ and H_2O extracted very little phosphorus in the soil. $CaCl_2$ extracted less than 1 mg kg^{-1} phosphorus in the soil, while H_2O extracted 0-2 mg kg^{-1} phosphorus. For the combined (average) topsoil, the Olsen method extracted higher (mean = 15.75 and 18.35 mg kg^{-1}, for sites with and without mortalities respectively) phosphorus compared to the $CaCl_2$ and H_2O extraction methods (Table 10). There was no trend over depth for the phosphorus extracted with the $CaCl_2$ and H_2O methods. A trend of phosphorus increasing with depth was, however, observed with the phosphorus extracted with the Olsen extraction method. Notably, the sites without mortalities had slightly higher Olsen phosphorus than the sites with mortalities (Table 9). There were no significant differences ($P>0.05$) observed between the methods used to extract phosphorus (Table 9).

5.2.4 pH water and KCl

The pH (H_2O) in the sites with mortalities had a minimal variation amongst all the depths with the topsoil having the lowest values. On the other hand, in sites without mortalities, a slight, statistical non-significant increasing trend of pH (H_2O) with increasing depth was observed. A similar trend in pH (KCl) was observed, where sites without mortalities had the lowest value in the topsoil and this increased with depth. However, the opposite behaviour was observed in pH (KCl) in the sites with mortalities, where the topsoil had the highest value and varied slightly within other depths (Table 9). For the combined topsoil, the pH (H_2O) and pH (KCl) in the sites with mortalities (mean = 8.23; 7.22) and those without mortalities (mean = 8.01; 6.96) did not differ significantly; ($P>0.05$) between sites with and without mortalities (Table 10).

5.2.5 Cation exchange capacity

Cation exchange capacity (CEC) in soil depths within the sites with mortalities did not display any clear trend, although the B2 horizon for sites with mortalities recorded the highest value of all the horizons (mean = 19.44 $cmol_c$ kg^{-1}; Table 9). In sites without mortalities, a slight decrease was noticed in CEC with an increase in depth (Table 9). The B2 horizon was the only depth in which a significant difference was identified, with the sites where RVF mortalities were reported having lower CEC (mean = 9.69 $cmol_c$ kg^{-1}) than in the sites where mortalities were not reported (mean = 19.44 $cmol_c$ kg^{-1}; Table 9). Sites with mortalities and sites without

mortalities did not vary significantly over the first three sampled depths (Table 10). It was, however, observed (Table 10) that CEC in the sites without mortalities was slightly higher (mean = 17.83 $cmol_c\ kg^{-1}$) than in sites with mortalities (mean = 16.28 $cmol_c\ kg^{-1}$).

5.2.6 Exchangeable cations

The exchangeable Ca^{2+} in the topsoil (Table 10) for sites without RVF mortalities was consistently higher (mean = 38.17 $cmol_c\ kg^{-1}$) than in the sites with mortalities (mean = 24.25 $cmol_c\ kg^{-1}$). There was also a non-significant increasing trend observed of exchangeable Ca^{2+} with an increase in depth in the sites with mortalities. Conversely, in sites without mortalities exchangeable Ca^{2+} was fluctuating across all depths (Table 10). Therefore, the sites with mortalities and sites without mortalities differed significantly ($P < 0.05$; Table 10).

The mean values of exchangeable K^+ were low in the topsoil (mean = 1.51 and 2.39 $cmol_c\ kg^{-1}$, for sites with and without mortalities respectively; Table 10); however, the first sampled layer had the highest values (mean = 1.73 and 2.62 $cmol_c\ kg^{-1}$, for sites with and without mortalities respectively; Table 10) and their means values declined with depth to 1.18 and 1.90 $cmol_c\ kg^{-1}$, for sites with and without mortalities respectively (Table 10). Notably, exchangeable K^+ in the sites without mortalities was approximately two times larger than in the sites with mortalities (Table 10). The differences in exchangeable K^+ between the two sites were statistically significant only in the topsoil and A horizons, with sites without RVF mortalities having higher values (mean = 2.37 $cmol_c\ kg^{-1}$) than sites with mortalities (mean = 1.50 $cmol_c\ kg^{-1}$; Table 10). The sites with mortalities and sites without mortalities did not differ significantly ($P > 0.05$), in exchangeable K^+ for the B1 and B2 horizons.

Exchangeable Mg^{2+} was higher in the sites without RVF mortalities, compared to the sites with mortalities. While there was no clear trend, Mg^{2+} accumulated on the B2 horizon therefore, making it have the highest Mg^{2+} content at all sites (mean = 9.58; 11.40 $cmol_c\ kg^{-1}$, for sites with and without mortalities respectively) while the A horizons had the lowest exchangeable Mg^{2+} (mean = 7.30; 10.95 $cmol_c\ kg^{-1}$; Table 10). The combined (average) topsoils were, however, significantly different ($P < 0.05$), and sites without mortalities had higher exchangeable Mg^{2+} (10.65 $cmol_c\ kg^{-1}$) than sites with mortalities (7.21 $cmol_c\ kg^{-1}$; Table 10).

For exchangeable Na^+, the mean values in the topsoils (Table 10) for the sites without mortalities were consistently higher (mean = 5.10 $cmol_c\ kg^{-1}$) than in the sites with mortalities (mean = 1.81 $cmol_c\ kg^{-1}$). Exchangeable Na^+ did not display any significant difference for all the depths (Table 10).

5.2.7 Soluble cations

At all sites, the mean values of soluble cations were all remarkably low in the soil. Notably, in sites without mortalities, soluble K^+ was not detected at all in the A and B1 horizons (Table 10). RVF mortality sites and sites without mortalities differed significantly for soluble Ca^{2+} and K^+ in all the horizons. On the other hand, soluble Na^+ and soluble Mg^{2+} did not differ significantly in any of the four sampled horizons (Table 10).

Table 9 Means of soil chemical properties, and their P values for mortality sites and sites without mortalities, per soil layer

Variables	Layers	Soil depth	Mortality sites	Sites without mortality	P-value
Organic Carbon (%)	1	Top soil	3.38	2.98	0.71
	2	A horizon	2.36	1.57	0.35
	3	B1 horizon	2.24	1.86	0.21
	4	B2 horizon	2.49	1.94	0.21
Total Nitrogen (%)	1	Top soil	2.23	2.45	0.82
	2	A horizon	2.07	1.24	0.90
	3	B1 horizon	1.67	6.93	0.38
	4	B2 horizon	2.70	5.37	0.53
Calcium Chloride ($CaCl_2$) Phosphorus (mg kg^{-1})	1	Top soil	0.37	0.35	0.48
	2	A horizon	0.21	0.18	0.77
	3	B1 horizon	0.28	0.45	0.56
	4	B2 horizon	0.22	0.38	0.18
Water extraction Phosphorus (mg kg^{-1})	1	Top soil	1.87	1.64	0.51
	2	A horizon	1.41	1.46	0.58
	3	B1 horizon	0.65	1.08	0.45
	4	B2 horizon	0.72	1.25	0.25
Olsen Phosphorus (mg kg^{-1})	1	Top soil	24.75	30.38	0.64
	2	A horizon	11.99	16.31	0.27
	3	B1 horizon	10.50	8.94	0.80
	4	B2 horizon	8.06	7.22	0.44
pH KCl	1	Top soil	7.31	6.62	0.18
	2	A horizon	7.16	6.97	0.42
	3	B1 horizon	7.18	7.29	0.98
	4	B2 horizon	7.20	7.49	0.26
pH water	1	Top soil	8.17	7.58	0.32
	2	A horizon	8.30	8.10	0.50
	3	B1 horizon	8.21	8.35	0.59
	4	B2 horizon	8.26	8.51	0.25
Cation Exchange Capacity (CEC) ($cmol_c$ kg^{-1})	1	Top soil	18.52	19.14	0.93
	2	A horizon	15.18	17.09	0.75
	3	B1 horizon	15.15	17.27	0.78
	4	B2 horizon	19.44	9.69	0.06*
Exchangeable Ca^{2+} ($cmol_c$ kg^{-1})	1	Top soil	22.43	37.87	0.01*
	2	A horizon	24.70	37.01	0.02*
	3	B1 horizon	25.60	39.65	0.02*
	4	B2 horizon	25.15	40.33	0.01*

Variables	Layers	Soil depth	Mortality sites	Sites without mortality	P-value
Exchangeable K^+ ($cmol_c\ kg^{-1}$)	1	Top soil	1.73	2.62	0.06*
	2	A horizon	1.38	2.35	0.04*
	3	B1 horizon	1.42	2.17	0.15
	4	B2 horizon	1.18	1.90	0.25
Exchangeable Mg^{2+} ($cmol_c\ kg^{-1}$)	1	Top soil	7.30	10.95	0.04*
	2	A horizon	6.80	9.78	0.03*
	3	B1 horizon	7.53	11.22	0.02*
	4	B2 horizon	9.58	11.40	0.21
Exchangeable Na^+ ($cmol_c\ kg^{-1}$)	1	Top soil	2.25	3.27	0.53
	2	A horizon	1.81	6.22	0.72
	3	B1 horizon	1.38	5.82	0.38
	4	B2 horizon	1.53	5.90	0.92
Soluble Ca^{2+} ($cmol_c\ kg^{-1}$)	1	Top soil	0.19	0.43	0.02*
	2	A horizon	0.23	0.40	0.02*
	3	B1 horizon	0.21	0.31	0.01*
	4	B2 horizon	0.17	0.27	0.02*
Soluble K^+ ($cmol_c\ kg^{-1}$)	1	Top soil	0.02	0.03	0.03*
	2	A horizon	0.00	0.03	0.00*
	3	B1 horizon	0.00	0.04	0.00*
	4	B2 horizon	0.00	0.03	0.00*
Soluble Mg^{2+} ($cmol_c\ kg^{-1}$)	1	Top soil	0.31	0.06	0.14
	2	A horizon	0.096	0.05	0.37
	3	B1 horizon	0.04	0.24	0.29
	4	B2 horizon	0.03	0.35	0.08
Soluble Na^+ ($cmol_c\ kg^{-1}$)	1	Top soil	6.32	0.59	0.63
	2	A horizon	0.95	1.18	0.43
	3	B1 horizon	0.33	1.39	0.09
	4	B2 horizon	0.18	1.34	0.10

* indicate statistical difference between the two groups with a p-value of 0.05

5.1 Texture

Descriptive statistics for soil particle size distribution for the sites where RVF mortalities were reported and sites, where they were not reported, are presented in Appendix C, while Table 11 gives the statistical comparison of the data. The textures vary from coarse sand to very fine sand and from coarse silt to clay. A general observation made was that the B2 horizon had the highest clay and this is due to clay illuviation in the soil profile. The topsoil clay content (Table 10) in sites without RVF mortalities was slightly higher (mean = 36.08%) than in sites with mortalities (mean = 31.08%). Conversely, at all sites, the sand fraction in the topsoil (Table 10) was predominantly fine, with the mortality sites having slightly higher fine sand (mean = 22.07%) than the sites without mortalities (mean = 17.75%). The second most dominant sand fraction was very fine sand and it presented a similar trend as that observed for fine sand. At less than 10%, the medium sand and coarse sand fractions were less dominant infrequent (Table 11). The mortality sites and sites without mortalities did not differ significantly in terms of the coarse, medium, and fine sand fractions. Very fine sand did not

differ significantly for the topsoil, A horizon, B1, and B2 horizons, when the mortality sites were compared to sites without mortalities (Table 10). A similar trend was observed for the coarse silt where the A horizon was the only depth that displayed a significant difference while the differences for all the other horizons were not significantly different (Table 11).

5.2 Salinity

5.2.1 Electrical Resistance

The topsoil at all sites had the highest electrical resistance in the soil (mean = 2187 Ω and 1924 Ω, for sites with and without mortalities respectively), which declined drastically with depth to a mean of 768 Ω and 580 Ω, for sites with and without mortalities respectively (Table 11). The electrical resistance of the saturated paste did not differ significantly between the mortality sites and sites without mortalities (Table 11).

5.2.2 Electrical conductivity

Converse to electrical resistance, the electrical conductivity (EC), varied considerably within the horizons across all sites. The topsoil had the lowest EC in both sites with mortalities and those without mortalities (mean = 20 mS m^{-1} and 14 mS m^{-1}, for sites with and without mortalities respectively). However, an increase with depth was observed in the sites without mortalities, while in mortality sites there was no such observable trend. No significant differences were observed between sites with reported RVF mortalities and sites without RVF mortalities (Table 11).

Table 10 Mean topsoil chemical and physical data, and their P values for all sites, calculated using the mean values per site for the first three soil layers, for sites with and without mortalities and their P values

Variables	Mortality sites	Sites without mortality	P-value
Organic Carbon (%)	2.66	2.14	0.37
Total Nitrogen (%)	1.99	1.46	0.83
Phosphorus (mg kg^{-1}) calcium chloride ($CaCl_2$) extraction	0.29	0.33	0.78
Phosphorus (mg kg^{-1}) water extraction	1.31	1.38	0.54
Phosphorus (mg kg^{-1}) Olsen extraction	15.75	18.35	0.67
pH KCl	7.22	6.96	0.48
pH water	8.23	8.01	0.59
Cation Exchange Capacity ($cmol_c\ kg^{-1}$)	16.28	17.83	0.72
Exchangeable Ca^{2+} ($cmol_c\ kg^{-1}$)	24.25	38.17	0.01*
Exchangeable K^+ ($cmol_c\ kg^{-1}$)	1.51	2.39	0.02*
Exchangeable Mg^{2+} ($cmol_c\ kg^{-1}$)	7.21	10.65	0.02*
Exchangeable Na^+ ($cmol_c\ kg^{-1}$)	1.81	5.10	0.87
Soluble Ca^{2+} ($cmol_c\ kg^{-1}$)	0.21	0.38	0.01*
Soluble K^+ ($cmol_c\ kg^{-1}$)	0.01	0.03	0.00*
Soluble Mg^{2+} ($cmol_c\ kg^{-1}$)	0.15	0.12	0.25
Soluble Na^+ ($cmol_c\ kg^{-1}$)	2.53	1.05	0.92
Clay (%)	31.08	36.08	0.35
Coarse sand (%)	4.18	3.15	0.53
Medium sand (%)	5.49	4.73	0.98
Fine sand (%)	22.07	17.75	0.34
Very fine sand (%)	14.27	10.34	0.11
Coarse silt (%)	6.81	9.16	0.05*
Resistance Ω	1427	1175	0.39
Electrical conductivity	64.09	21.51	0.156

* indicate statistical difference between the two groups with a p-value of 0.05

Table 11 Means of soil physical properties of mortality sites and sites without mortality

Variables	Layers	Soil horizon	Mortality sites	Sites without mortality	P-value
Clay (%)	1	Top soil	30.0	34.2	0.40
	2	A horizon	27.4	35.4	0.13
	3	B1 horizon	36.0	38.6	0.69
	4	B2 horizon	39.6	41.5	0.77
Coarse sand (%)	1	Top soil	3.98	3.86	0.92
	2	A horizon	4.04	2.49	0.50
	3	B1 horizon	4.50	3.11	0.87
	4	B2 horizon	3.23	3.22	0.55
Medium sand (%)	1	Top soil	4.99	4.66	0.72
	2	A horizon	6.03	4.79	0.75
	3	B1 horizon	5.46	4.74	0.96
	4	B2 horizon	3.84	3.77	0.55
Fine sand (%)	1	Top soil	22.6	16.9	0.22
	2	A horizon	24.8	18.6	0.19
	3	B1 horizon	18.8	17.7	0.73
	4	B2 horizon	18.1	13.9	0.36
Very fine sand (%)	1	Top soil	14.2	10.0	0.075
	2	A horizon	16.8	11.2	0.076
	3	B1 horizon	11.8	9.8	0.22
	4	B2 horizon	12.0	9.8	0.29
Coarse silt (%)	1	Top soil	9.0	8.0	0.21
	2	A horizon	5.4	8.9	0.03*
	3	B1 horizon	7.0	8.8	0.18
	4	B2 horizon	7.6	8.6	0.39
Resistance Ω	1	Top soil	2187	1924	0.60
	2	A horizon	1165	958	0.35
	3	B1 horizon	928	642	0.22
	4	B2 horizon	768	580	0.46
Electrical Conductivity (mS m^{-1})	1	Top soil	20	14	0.31
	2	A horizon	138	19	0.18
	3	B1 horizon	34	32	0.74
	4	B2 horizon	21	36	0.43

* indicate statistical difference between the two groups with a p-value of 0.05

5.3 Mineralogical analysis

5.3.1 Major elements

SiO_2, Al_2O_3, CaO, and Fe_2O_3 were the most abundant major elements found at all sites, while MnO was the least abundant. Other elements found were in small quantities (Figure 15). There were no significant differences between the mortality sites and sites without mortalities for any of the major elements (Table 12).

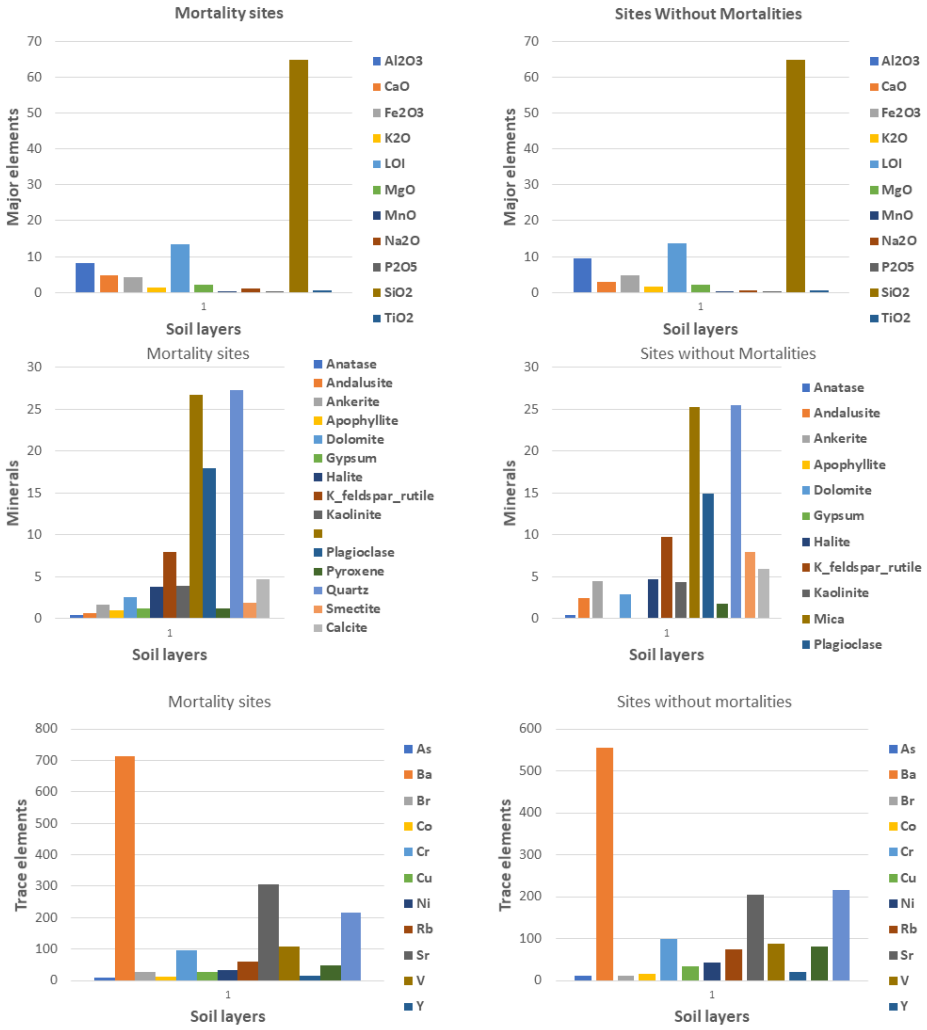


Figure 15 Mineralogical and chemical characterisation of sites with mortalities and sites without mortalities

Table 12 Means of macro elements in the topsoils of the sites with and without mortalities and their P values

Compound	Mortality Sites	Sites without mortalities	P-value
Al_2O_3 (%)	8.08	9.42	0.20
CaO (%)	4.90	2.92	0.21
Fe_2O_3 (%)	4.37	4.76	0.44
K_2O (%)	1.40	1.67	0.08
LOI (%)	13.57	13.70	0.85
MgO (%)	2.23	2.19	0.86
MnO (%)	0.05	0.08	0.14
Na_2O (%)	1.01	0.69	0.24
P_2O_5 (%)	0.09	0.12	0.10
SiO_2 (%)	64.91	64.69	0.76
TiO_2 (%)	0.52	0.50	0.77

* indicate statistical difference between the two groups with a p-value of 0.05

5.3.2 Minor elements

Ba was the most abundant trace element at all sites (Table 13). Other elements varied in different quantities between the sites. In the mortality sites, the second most abundant element was Sr followed by Zr, V, and Cr respectively. Whereas in sites without mortalities, Zr was the second most abundant element, followed by Sr, Cr, and V respectively. Other elements including As, Co, Cu, Ni, Rb, Y, Zn, Ba, Br, Sr, and V were observed in small quantities in the soil (Figure 15).

Mortality sites and sites without mortalities yielded significant differences in Ba, Ni, Y, and Zn (Table 13). Mortality sites had higher Ba and lower Ni, Y, and Zn, than sites without mortalities. There were no significant differences in As, Br, Co, Cr, Cu, Rb, Sr, V, and Zr in both mortality sites and sites without reported mortalities.

Table 13 Means minor-elements in the topsoils of the sites with and without mortalities and their P values

Element	Mortality sites	Sites without mortalities	P-value
As mg kg^{-1}	8.25	10.80	0.19
Ba (mg kg^{-1})	712.00	554.86	0.03*
Br (mg kg^{-1})	26.71	12.01	0.06
Co (mg kg^{-1})	11.77	14.99	0.20
Cr (mg kg^{-1})	95.57	98.61	0.95
Cu (mg kg^{-1})	28.12	34.83	0.15
Ni (mg kg^{-1})	32.04	42.84	0.03*
Rb (mg kg^{-1})	59.22	73.62	0.09
Sr (mg kg^{-1})	305.17	204.39	0.68
V (mg kg^{-1})	108.71	88.05	0.24
Y (mg kg^{-1})	15.74	21.53	0.02*
Zn (mg kg^{-1})	49.57	82.12	0.06*
Zr (mg kg^{-1})	216.43	216.59	0.75

* indicate statistical difference between the two groups with a p-value of 0.05

5.3.3 Mineralogy of the clay fraction

Quartz was by far the most dominant mineral in all sites (mean = 27.33% and 25.45%, for sites with and without mortalities respectively). Mica was the second most abundant mineral (mean = 26.76% and 25.29%, for sites with and without mortalities respectively) in the clay fraction and only slightly lower than quartz. The third most abundant mineral was plagioclase (mean = 17.94 and 14.99%, for sites with and without mortalities respectively). The rest of the minerals were present only in small quantities (<10%). It was interesting to note that smectite was four times higher in sites without mortalities (mean = 7.99%) when compared to mortality sites (mean = 1.96%). Minerals that include apophyllite and gypsum were not detected at all in the sites without mortalities, whereas it was detected in small quantities (mean = 0.96%) in the mortality sites (Table 14). There were, however, no significant differences in the clay

mineralogy between the sites with reported RVF mortalities and sites without RVF mortalities (Table 14).

Table 14 Mean mineralogy of the clay fraction of the topsoils of sites with and without mortalities and their P values

Minerals	Mortality sites	Sites without mortalities	P-value
Anatase (%)	0.43	0.43	0.91
Andalusite (%)	0.68	2.45	0.35
Ankerite	1.70	4.45	0.26
Apophyllite (%)	0.96	0.00	0.41
Calcite (%)	4.66	5.90	0.60
Dolomite (%)	2.62	2.94	0.89
Gypsum (%)	1.23	0.00	0.41
Halite (%)	3.84	4.71	0.79
K_feldspar_rutile (%)	7.94	9.81	0.95
Kaolinite (%)	3.88	4.41	0.85
Mica (%)	26.76	25.29	0.84
Plagioclase (%)	17.94	14.99	0.10
Pyroxene (%)	1.25	1.74	0.91
Quartz (%)	27.33	25.45	0.53
Smectite (%)	1.96	7.99	0.14

* indicate statistical difference between the two groups with a p-value of 0.05

5.4 Soil classification

The mortality sites were dominated by Kastanozems and Calcisols, with Calcic, Protostagnic, and Hypocalcic qualifiers. Site p002bulwltv was the only one characterised by Vertisols, while p003bulmgkr was characterised by as a Stagnosol. In the sites without mortalities Calcisols dominated. The new sites without reported mortalities were dominated by Kastanozems, Calcisols, Luvisols, and Stagnosols (Table 15).

Table 15 Soil classification of sites with and without mortalities using the South African Taxonomy and World Reference Base (IUSS Working Group WRB, 2015)

Farm ID	Depth (mm)	Texture	Soil form	Soil family	WRB	Mortalities
p013bradlpn	1500	Sandy clay	Montagu	1110	Calcic Kastanozem (Loamic, Cambic, Stagnic)	Reported
p001brawtkr	600	Sandy clay	Montagu	1100	Calcic Kastanozem (Anthric, Clayic, Pachic, Stagnic)	Reported
p002bulwltv	910	Clay	Rensburg	1000	Haplic Vertisol (Mollic, Stagnic)	Reported
p004bullmrl	900	Sandy loam	Montagu	1110	Protostagnic Kastanozem (Loamic, Stagnic)	Reported
p009deaqwgg	1100	Clay	Montagu	1110	Haplic Calcisol (Clayic, Hypercalcic, Stagnic)	Reported
p009deaqwgg	960	Clay	Montagu	1110	Hypocalcic Kastanozem (Clayic)	Reported
p011petmrtn	900	Clay	Montagu	1110	Hypocalcic Kastanozem (Clayic)	Reported
p010jacrtrv	530	Sandy loam	Westleigh	1000	Haplic Calcisol (Loamic, Hypercalcic)	Reported
p008oppdmsh	1200	Clay loam	Montagu	1100	Haplic Calcisol (Clayic, Hypocalcic, Stagnic)	Reported
p007lucetrp	630	Loamy sand	Augrabies	2100	Haplic Calcisol (Arenic, Hypercalcic, Stagnic)	Reported
p005petbrkp	630	Sandy loam	Tukulu	1110	Hypocalcic Kastnozem (Loamic, Cambic, Oxyaquic)	Reported
p012redbgnv	700	Clay loam	Augrabies	1110	Haplic Kastnozem (Clayic, Chromic)	Reported
p003bulmgkr	1000	Sandy clay loam	Katspruit	2000	Calcic Mollic Stagnosol (Loamic)	Not reported
p014blo7dms	540	Sandy loam	Klapmuts	1120	Cambic Mollic Stagnosol (Loamic)	Not reported
p006bftddmm	560	Sandy clay	Addo	1111	Haplic Umbrisol (Clayic, Pachic)	Not reported
p006bftddmm	520	Sandy clay	Brandvlei	2000	Haplic Calcisol (Loamic, Hypercalcic)	Not reported
p015kimgrsp	1800	Sandy clay	Katspruit	1000	Haplic Stagnosol (Loamic)	Not reported
p015kimgrsp	1600	Sandy clay	Brandvlei	2000	Haplic Calcisol (Loamic, Hypercalcic)	Not reported
p015kimgrsp	1500	Sandy loam	Brandvlei	2000	Haplic Calcisol (Loamic, Hypercalcic, Protostagnic)	Not reported
p015kimgrsp	1500	Sandy clay	Brandvlei	2000	Haplic Calcisol (Loamic, Hypercalcic)	Not reported
p015kimgrsp	1700	Loam	Augrabies	1100	Haplic Calcisol (Loamic, Hypercalcic)	Not reported
p015kimgrsp	1100	Loam	Brandvlei	2000	Haplic Calcisol (Loamic, Hypocalcic)	Not reported

Farm ID	Depth (mm)	Texture	Soil form	Soil family	WRB	Mortalities
321hoppplr	1200	Clay loam	Addo	2112	Calcic Luvisol (Clayic, Ochric)	Not reported
222phibthl	1200	Silty clay	Addo	1112	Calcic Luvisol (Clayic, Ochric)	Not reported
259edemdws	1200	Silty clay	Longlands	1000	Albic, Pisoplinthic Luvisol (Clayic Ochic)	Not reported
140troklkn	1000	Clay	Augrabies	1100	Protocalcic Luvisol (Clayic Ochric)	Not reported
211bosxcls	700	Sandy clay loam	Augrabies	1110	Protocalcic Luvisol (Clayic Ochric)	Not reported
284petkrfs	1200	Clay	Augrabies	1210	Protocalcic Luvisol (Clayic Ochric)	Not reported
131smibssv	1200	Clay	Longlands	1000	Stagnic, Pisoplinthic Luvisol (Clayic Ochric)	Not reported
131smibssv	1200	Clay	Kastpruit	1000	Dystric, Luvic, Reductic Stagnosol (Clayic, Ochric)	Not reported

5.5 Discussion

The CEC only displayed statistical significance in the 4th layer where mortality sites had the highest CEC than sites without mortalities. This, however, did not influence the mosquito egg and the virus since the *Aedes* mosquito deposits its eggs 60 mm below the soil surface (Jupp, 2005). Conversely, the exchangeable Ca^{2+} in all the four horizons, exchangeable K^{+} in the A and B horizon, exchangeable Mg^{2+} in all horizons except B2; displayed a significant difference, while exchangeable Na^{+} was the only one that did not show any significant difference. Soluble cation concentrations were low in the soil although a statistically significant difference was observed in soluble Ca^{2+} and soluble K^{+}. For all these cations, the sites without reported RVF mortalities had a higher mean value, while sites with reported RVF mortalities' mean values were lower. A similar trend was observed in the study conducted by Verster (2016) where soluble Ca^{2+}, exchangeable Ca^{2+}, exchangeable Mg^{2+}, exchangeable $K^{+,}$ and CEC were statistically significant with high mean values in the sites without RVF mortalities and low in the sites with RVF mortalities. These properties are, therefore, suspected to have played a role in the outbreaks of RVF.

In this study, the soil resistance was considerably higher in the topsoil and decreased with depth. The electrical resistance in sites with reported mortalities was slightly higher than in sites without mortalities. Salinity was also reported to play a role in promoting the abundance of mosquitoes (Baba *et al*., 2016; Ofulla *et al*., 2010). Salts are reported to encourage the growth of mosquito larvae (Clark *et al*., 2004).

Few studies that associate texture to RVF epidemics have been conducted and it was reported that clay soil was the most relevant texture to favour RVF outbreaks. Baba *et al*. (2016),

indicate that the Rift Valley, where the disease was initially observed, is characterised by clay and clay loamy soil textures. In South Africa, Brand *et al.* (2018) conducted a visual field study where it was observed that in the epicentre of the 2010 RVF outbreak, that in the Free State and Northern Cape Provinces, high clay content was common in sites where RVF mortalities were reported compared to sites without RVF mortalities. Unlike sandy soils, soils high in clay and loam have a high water holding capacity meaning they can retain water for a longer period. This could lead to a more prolonged saturation, become inundated and therefore, promote flooding. Upon flooding, a conducive environment is created for the breeding and survival of the floodwater *Aedes* mosquito vectors through the accumulation of salts. In mortality sites, 75% of the sites are dominated by clay and clay loam, whereas the remaining 25% are dominated by sand (loamy sand, sandy loam, and sandy clay loam). In the old sites without mortalities, by contrast, sand (sandy clay loam, sandy clay, loam sand, and sandy loam) predominates. In the new sites without mortalities, clay and clay loam predominate. Mortality sites and new sites without RVF mortalities have better water retention when compared to the old sites without mortalities. Remarkably, in this study, coarse silt was found to be significantly different only in the A horizon with a high mean in sites without mortalities as compared to mortality sites. Coarse silt has a lower water holding capacity than clay and can thus result in drier environments that do not favour mosquito eggs' aestivation. Conversely, in the study of Verster (2016), medium sand was found to be significantly different between mortality sites and sites without mortalities (i.e. mortality sites had higher medium sand which can, therefore, indicate good retention capacity).

Kastanozems and Calcisols were widely reflected in the sites with reported mortalities. These soil types are largely confined to arid and semi-arid climate areas. They are characterised by the accumulation of secondary lime ($CaCO_3$) and other carbonates (IUSS Working Group WRB, 2015). The soils are formed through limited leaching, occurring through various factors, such as limited rainfall, high evapotranspiration or it could be due to sublayers high in clay which restrict permeability (Driessen *et al*., 2001). These soil types are all calcareous and, therefore, the presence of lime and carbonates could indicate the potential to adsorb and retain soil water (Hennessy *et al*., 1983). It could also be an indication and evidence of previous stagnant water or a fluctuating water table (Netterberg, 1978). The pH in these soils was greater than seven (7) and vary throughout the profile. Therefore, the alkalinity in these soils could also possibly indicate the presence of free lime or carbonates which indicate freestanding water. Vertisols are characterised by heavy clay with swelling and shrinking properties, thus allowing for long periods of water stagnation. Mollic Stagnosol and Mollisols have a high base saturation [base saturation is the proportion (expressed as a percentage) of the cation exchange capacity that is saturated with potassium (K), calcium (Ca), magnesium

(Mg) and sodium (Na)], and a high accumulation of organic matter. Both these are characteristic of soils with poor drainage capacity. And lastly, Stagnosols are characterised by signs of wetness (low chroma colours, clay coatings, mottles, and concretions). Conversely, the old sites without mortalities were dominated by Haplic Calcisols. The only difference was that mortality sites were more clayey and were dominated by stagnic properties, which could indicate that water was saturated for a longer period as compared to sites without mortalities, which were dominated by loamy soil with good soil drainage. The new sites without reported mortalities were characterised by clay soils, signs of wetness, and accumulation of secondary carbonates.

Barium ranged from 100 to 3000 mg kg^{-1} in natural soils (Choudhury *et al*., 2011). In this study, Ba ranged between 712.00 mg kg^{-1} in mortality sites and 554.86 mg kg^{-1} in sites without mortalities The differences between the sites with RVF mortalities and sites without mortalities might have been due to different human activities near the sites, anthropogenic inputs and different geology (Ong *et al*., 2013; Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, 2013). While Choudhury *et al*. (2001) suggest Ba can be mobile in the soil, ATSDR (2007) suggested that Ba mobility depends on the soil characteristics. Therefore, it is possible that in sites without RVF mortalities reported, Ba might have leached downwards to the subsoils. Ba is influenced by the clay content in the soil and can be adsorbed to the clay minerals, organic and fine structured soil (Ong *et al*., 2013; Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, 2013).

Nickel, Y, and Zn are reported by Wuana & Okieimen (2011) to be toxic elements in the soil. These elements can, thus, affect the microbial activity and microbial population in the soil (Bansal, 2018). Toxic elements can also contaminate the soil and result in changes in the chemical and physical properties (Bansal, 2018). In sites without reported RVF mortalities, these elements were higher than in the mortality sites, where they might have caused a decrease in microorganisms and might have also suppressed the RVFV because they are toxic. In the study conducted by Verster (2016), Bo and As were found to be significantly higher in sites without mortality than in mortality sites. It was speculated that As might have suppressed the virus in sites without mortality, but there was no hypothesis offered for Bo.

5.6 Conclusions

This study intensively investigated the soil properties that can have an impact on *Aedes* mosquitoes and RVFV. Soil properties include cations such as exchangeable Ca^{2+}, $K^{+,}$ and Mg^{2+}, Soluble Ca^{2+} and K^{+}, coarse silt, Ba, Ni, Y, and Zn. The mean of these properties was higher in sites without mortalities and low in mortality sites. Coarse silt and Ba had means

higher in mortality sites than in sites without mortality. Soils in sites with mortalities and new sites without mortalities were all characterised by soils that can retain water for longer than old sites with mortalities.

Chapter 6: Microbial activity, soil water levels, and mosquito abundance

6.1 Introduction

Microorganisms in the soil play a significant role in some of the ecological processes that sustain life. These processes include nutrient cycling, the decay of plant matter, consumption, and production of trace gases, and the transformation of metals (Panikov, 1999). Soil functioning, together with soil microbial community is greatly influenced by climate, especially precipitation and temperature (IPCC, 2007). Shifts in precipitation can result in an increase and decrease in soil water regimes such as during flooding and drought periods. Soil moisture resulting from precipitation, which is the most important environmental factor, influences soil microbial growth and activity. For example, it can result in a decrease or increase of microbial community and activity (Newton *et al*., 1939; Campbell & Biederbeck, 1976).

Soil temperature is another environmental factor influencing the proportion of soil microbes and temperatures that are too cold or too warm will inhibit microbial growth. The variation of soil microbes in a soil profile is influenced by ambient temperatures or seasonal changes. This has been widely reported by, for example, Bath & Soderstrom (1982) who indicated that winter and summer periods associate well with low soil microbial activities, whereas high microbial activities are typically recorded towards the end of spring and autumn. Conversely, Kaiser & Heinemeyer (1993) found microbial activities to be high in summer when temperatures were high and there were low activities in the winter months. A further contrast noted by van Gestel *et al.* (1992), indicated that winter months are associated with high moisture conditions resulting in high microbe numbers, whereas summer seasons are associated with low microbial abundance because it is typically dry. Holmes & Zak (1994), observed no relationship between microbial activity and seasonal changes.

Mosquito species that include *Aedes, Culex, Anopheles Eretmapodites,* and *Mansonia* are known to be efficient vectors of many viruses. Rift Valley fever, in particular, is primarily transmitted by the *Aedes* mosquitoes. *Culex* and *Anopheles* are the secondary vectors of this virus. In southern Africa, five mosquito species are known vectors for the RVF. These include *Aedes circumluteolus*, *A. mcintoshi*, *A. juppi*, *Culex theileri,* and *C. zombaensis* (Jupp, 2005). The virus is maintained in inter-epidemic periods through transovarial transmission in floodwater *Aedes* mosquitoes. *Pans* and *vleis* (known as *Dambos* in East Africa) are known to be conducive for breeding of these mosquitoes (Linthicum *et al*., 2016).

This chapter aims to address the second objective of this study, namely to estimate seasonal microbial activity at the research sites in relation to soil water levels, mosquito abundance, and species.

6.2 Results

6.2.1 Fluorescein diacetate (FDA)

The results displayed a great seasonal variation and differences between the sites when comparing microbial activity at the sites with mortalities between 2015 (Verster, 2016) and 2018. In sites like p013bradlpn, p001brawtkr, and p002bulwltv, the microbial activity did not vary considerably seasonally. On the other hand, sites like p009deaqwgg#1, p009deaqwgg#2, p011petmrtn, p010jacrtrv, and p005petbrkp had the highest microbial activity in 2015 and the lowest in 2018. Additionally, other sites had the highest microbial activity in 2018 and the lowest in 2015, including p004bullmrl, p008oppdmsh, p007lucetrp, and p012redbgnv (Figure 16).

In sites without mortalities, p003bulmgkr and p015kimgrsp#2 had a minimal difference in microbial activity between the years. p006bftddmm#1, p015kimgrsp#3, p015kimgrsp#4, p015kimgrsp#5, and p015kimgrsp#1 had the highest microbial activity in 2015 and it was minimum in 2018. p006bftddmm#2 and p015kimgrsp#6 were the only two sites that recorded high microbial activities compared to other sites in 2018 compared to 2015 (Figure 17).

In new sites without RVF mortalities, 211bosxcls had the highest microbial activity in the soil followed by 321hoppplr, 131smibssv#1, 140troklkn and 222phibthl respectively. All the remaining sites (284petkrfs and 259edemdws) had the microbial activity lower than 20 with 131smibssv#2 having the lowest value (Figure 18).

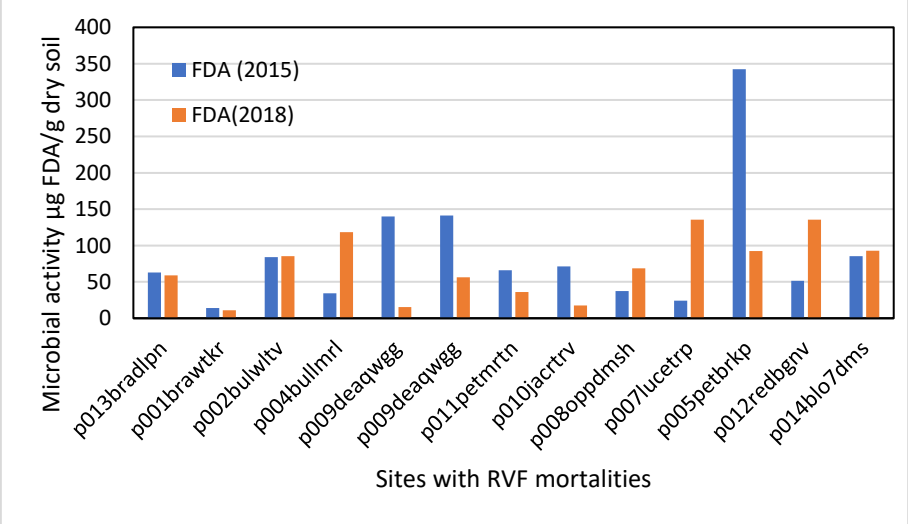


Figure 16 Comparison of the 2015 and 2018 microbial activity results for sites with reported RVF mortalities.

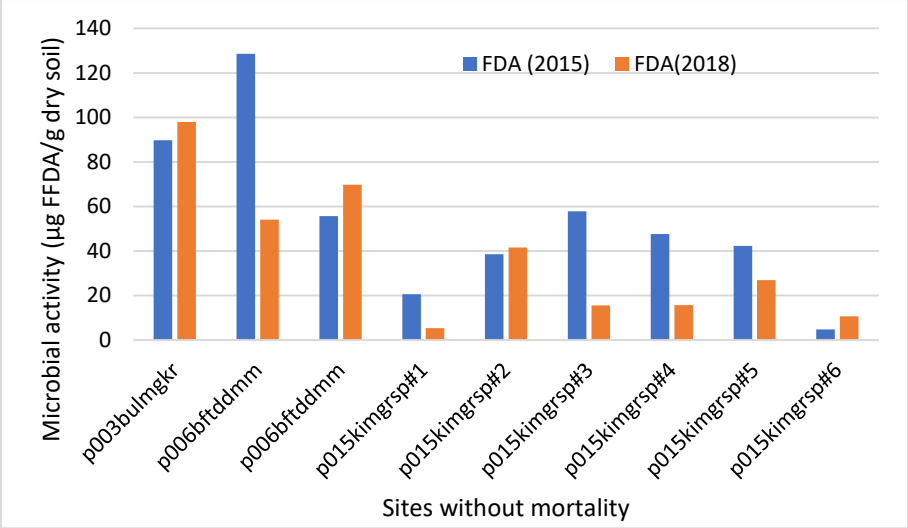


Figure 17 Comparison of the 2015 and 2018 microbial activity results for sites without reported RVF mortalities.

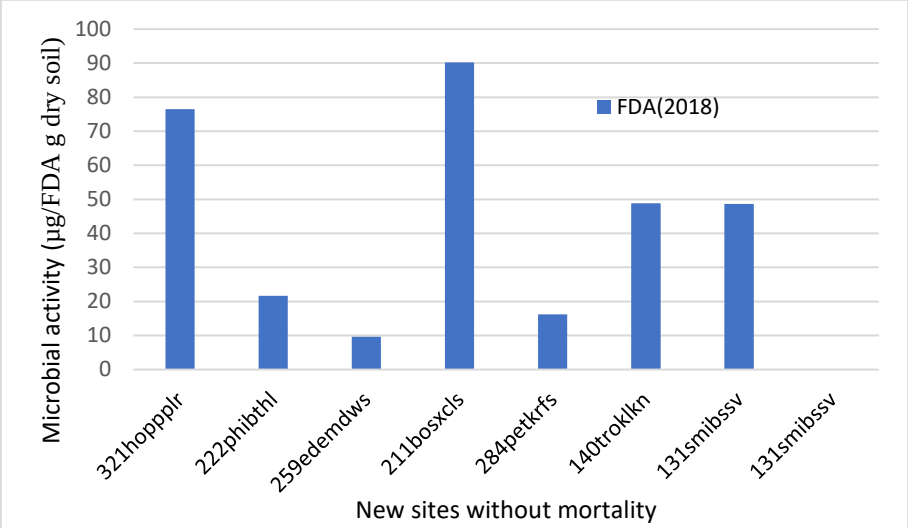


Figure 18 The 2018 microbial activity results for the new sites (no measurements were made in 2015) without reported RVF mortalities.

Statistical tests were carried out to compare the sites with mortality data between 2015 and 2018, sites without mortalities between 2015 and 2018, and lastly, tests between all the sites combined. The test used was the paired t-test. The mean differences between the old sites and new sites were not statistically significant. Although it was noticed that there were individual sites where the 2015 values were much larger than 2018 values, the reverse was also true. There were some sites where the 2015 values were smaller than the 2018 values. Microbial activity, therefore, did not differentiate between sites with and without reported RVF mortalities.

Table 16 Statistical test results for microbial activity, comparing all sites with mortalities and sites without mortalities between 2015 and 2018 period

Sites	Mean	paired t-test
Sites with mortalities between (2015/2018)	19.88	0.5082
Sites without mortalities between (2015/2018)	16.45	0.1266
All sites	18.41	0.2857

6.2.2 Water data

6.2.2.1 Level logger data

6.2.2.1.1 Sites with reported RVF mortalities

The level loggers were installed in different depths in the study sites due to different limiting rocks. They were initially installed in 2015 and during this period South Africa was under drought. Therefore, the soil moisture levels were at 0m indicating that the soil is dry. Some sites represented a negative value indicating that the soils were excessively dry. The level loggers reached full saturation at a value of 3m. There were generally gaps in the data due to technical problems of level loggers and level loader, loss of loggers, installing new ones, and animal damage. All these resulted in data loss and thus caused gaps.

Figure 19 shows that between 2015 and the beginning of 2017, a constant water level was observed. During this period, only a few (less than 162 counts) mosquitoes were observed. This was attributed to the drought that occurred in South Africa during that period. The mosquito count of 3415 that occurred in 2015 represented an outlier. From 2017 to the first quarter of 2018, a steady increase in water level was noted. This resulted in an increase of mosquito count up to a maximum of 1223. From the first quarter of 2018 onwards, a great fluctuation in both water level depth and mosquito count existed. The variation between the availability of water and mosquito count indicated that one follows the other very well.

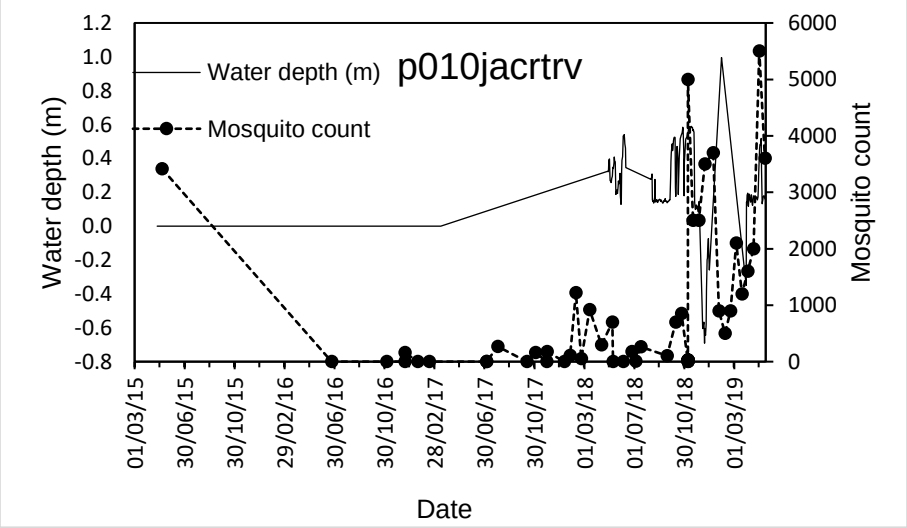


Figure 19 Relationship between water level depth and mosquito count in p010jacrtrv.

Between 2015 and 2016 at p001brawtkr (Figure 20), the water level was 0 m and there were only 13 mosquitoes collected during this period. A drastic increase in water level was observed from the beginning of 2017. During this period, fluctuations in water level were observed between summer and winter months. The mosquito count was low. A drastic increase in mosquito count was noted in the first quarter of 2018 and 2019 with a maximum mosquito count of 903.

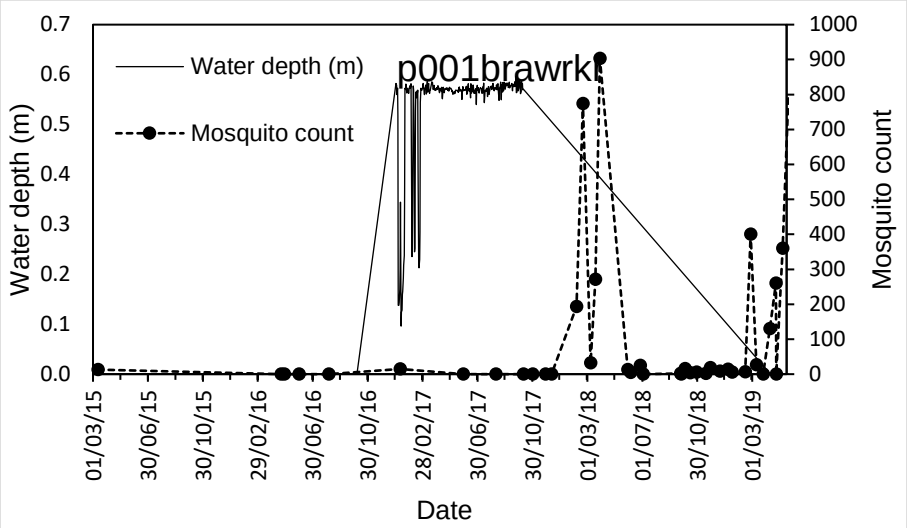


Figure 20 Relationship between water level depth and mosquito count in p001brawtkr.

At p009deaqwgg#1 (Figure 21), the water level remained above 0 m and fluctuated gradually between the years. The mosquito count was initially high from March 2015 ranging between 100 and 200 but decreased drastically until it reached 0 between May 2016 until December 2016. From January 2017 until June 2017, the mosquito count was between 200 and 300 and the water level was about 1.6 m. From February to June 2018, the mosquito count ranged between 100 and 600. Irregular rising and falling in water levels and mosquito count was noticed between June 2018 and May 2019.

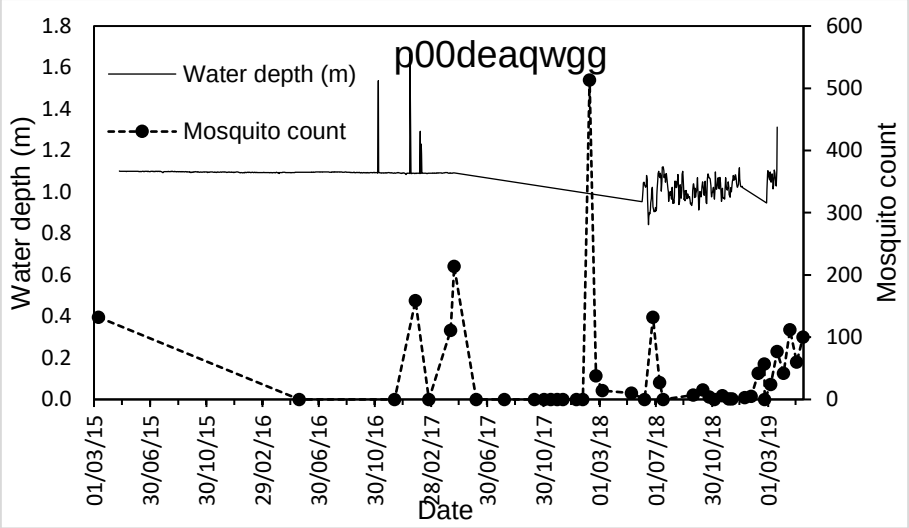


Figure 21 Relationship between water level depth and mosquito count in p009deaqwgg.

For p009deaqwgg #2 (Figure 22), the water level was above 0 m and fluctuated between the years. The mosquito count was initially high from March 2015 ranging between 100 and 200 but decreased drastically until it reached 0 in May 2016 until December 2016. From January 2017 until June 2017, the mosquito count was between 200 and 300 and the water level still fluctuated between 0.8 and 1.0 m. From February to June 2018, the mosquito count ranged between100 and 600. Water level (0.8-1) and mosquito count (5-132) displayed a fluctuation from June 2018 to May 2019.

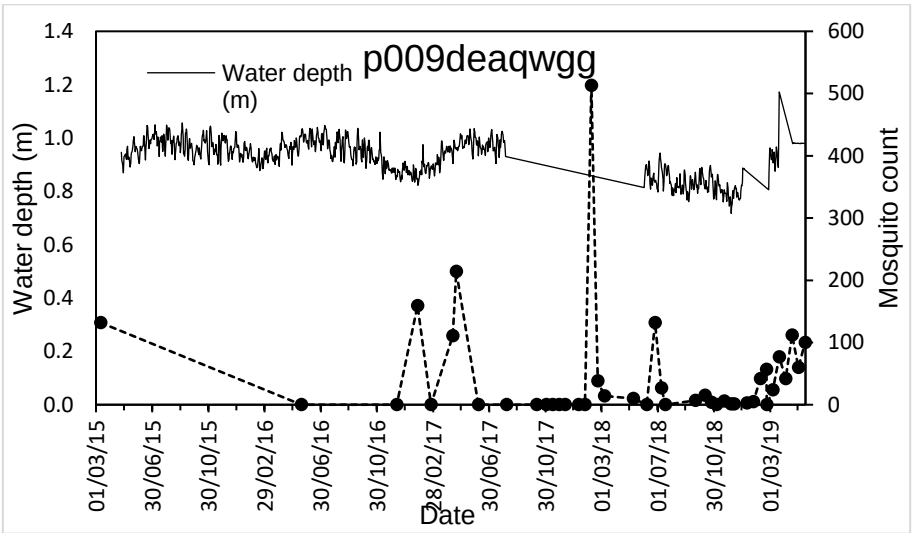


Figure 22 Relationship between water level depth and mosquito count in p009deaqwgg.

In p011petmrtn (Figure 23), the water level fluctuated between -0.01 m and 1.1 m. This, however, did not cause an impact on variability in mosquito count. The mosquito count varied between October 2015 until April 2017. A low mosquito count was observed between June 2017 and January 2018. From February 2018, there was a drastic increase in mosquito count and it reached a maximum of 493 in April 2018. A decrease in mosquito count was observed

in winter months when no rainfall was received. A mosquito count of 120 was obtained in March 2019.

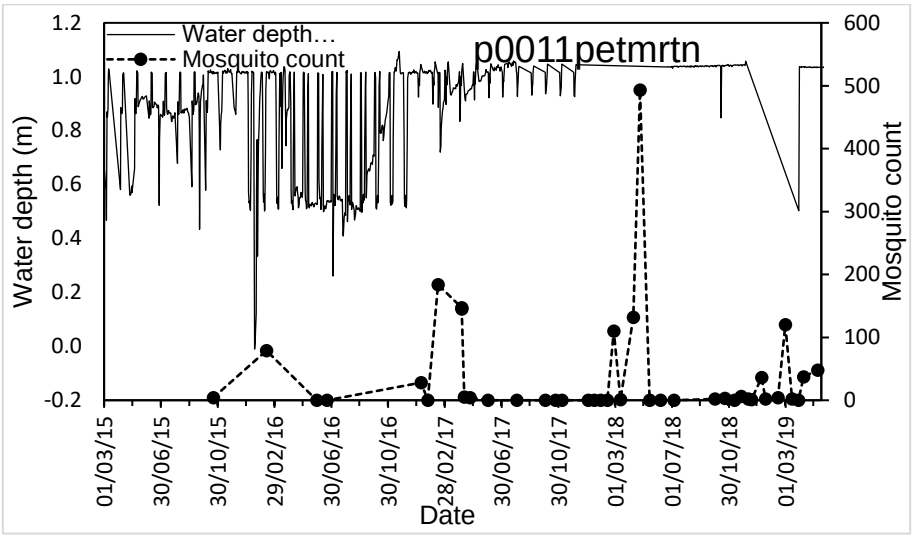


Figure 23 Relationship between water level depth and mosquito count in p011petmrtn.

At p002bulwltv (Figure 24), the water level was constant between August 2015 and August 2016 and the mosquito count was between 0 and 61. Fluctuation in water level and mosquito count was observed between October 2016 and January 2017 and again in the second quarter of 2018 and the beginning of 2019.

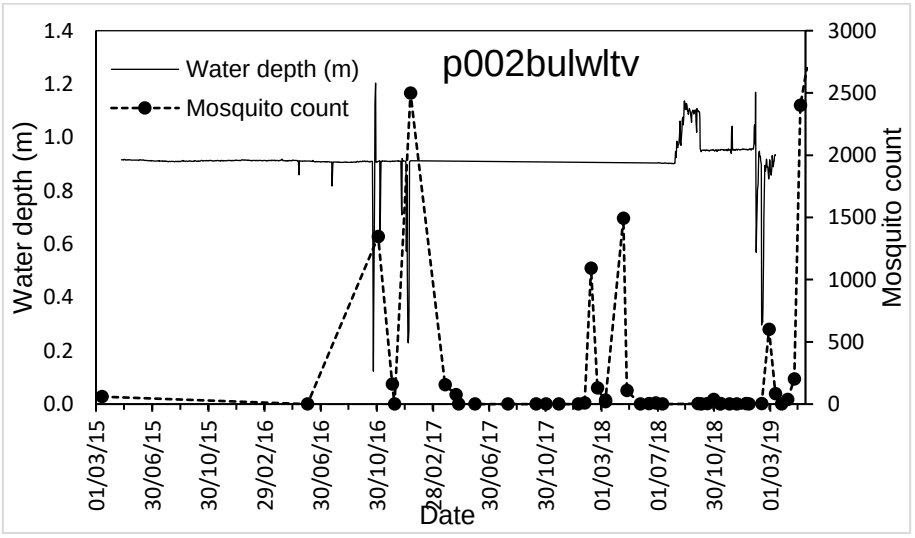


Figure 24 Relationship between water level depth and mosquito count in p002bulwltv.

For p008oppdmsh (Figure 25), the water level fluctuated between 0.1 and 1.7 m. The mosquito count was low and started to increase from February 2018 and reached a maximum of 2000 in April 2018. An increase in mosquito count was noted in 2019, associated with a fluctuating water level.

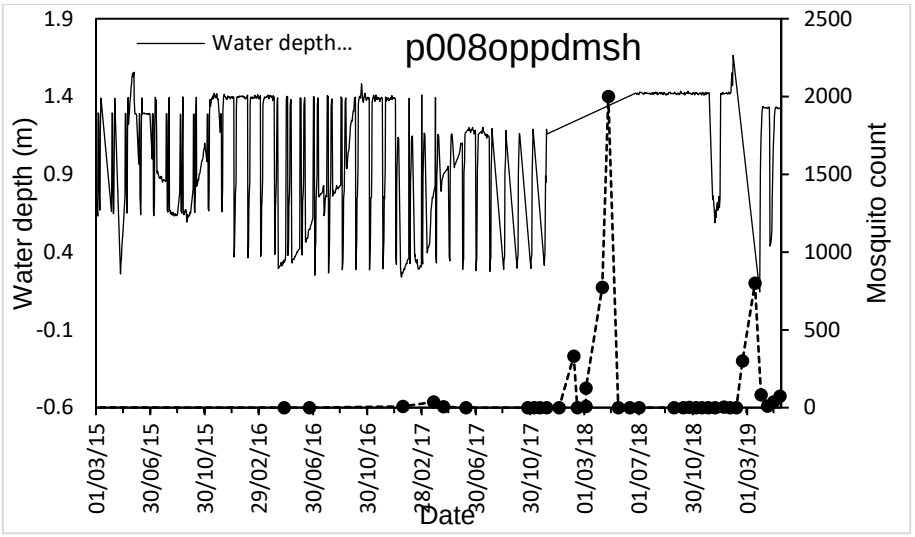


Figure 25 Relationship between water level depth and mosquito count in p008oppdmsh.

6.2.2.1.2 Sites without RVF mortalities

At p015kimgrsp (Figure 26), the water level fluctuated between 0.1 and 2.3 m throughout the profile. Mosquito count on the other hand slightly went up to 76 in February 2016 and thereafter went down to 0. A drastic increase to 334 was noted in February 2018. Towards the end, a great fluctuation on both water level and mosquito count was observed.

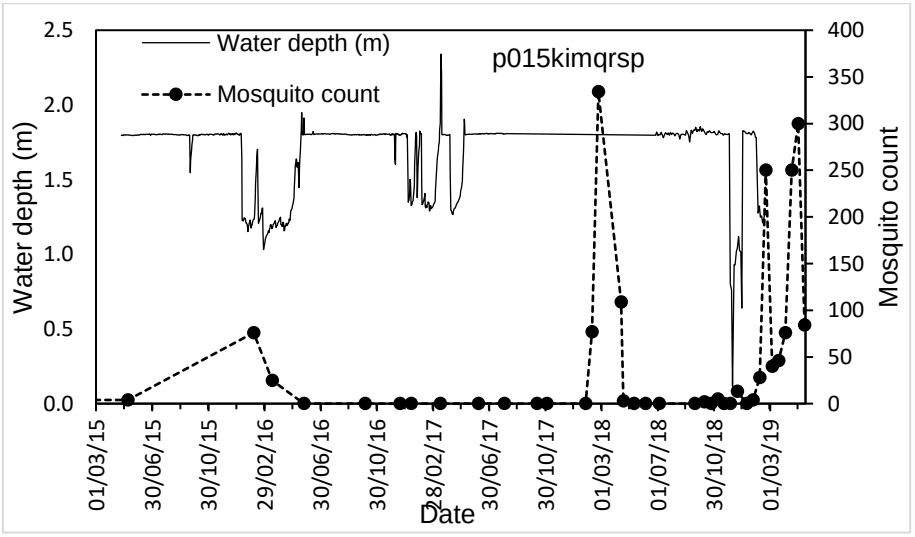


Figure 26 Relationship between water level depth and mosquito count in p015kimgrsp.

For site p006bftddmm (Figure 27), a great fluctuation of water level was observed between –0.19 and 1 m. The mosquito count was high in February 2017 (663) and January 2019 (1650) following a slight increase in water level.

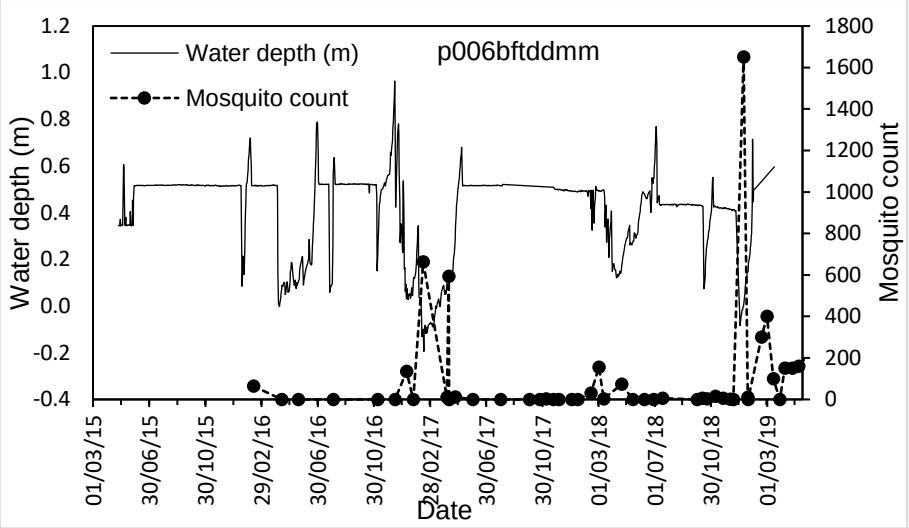


Figure 27 Relationship between water level depth and mosquito count in p006bftddmm.

At site p003bulmgkr (Figure 28), the water level was between 1 and 1.2 m. An increase in mosquito count was observed in the summer months of March 2017 (627), April 2018 (499), and May 2019 (1100).

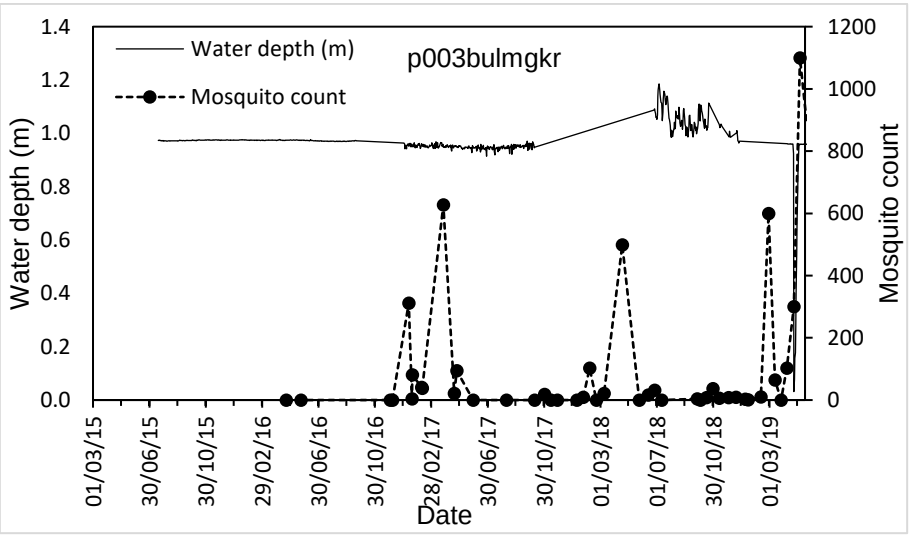


Figure 28 Relationship between water level depth and mosquito count in p003bulmgkr.

6.2.2.1.3 New sites without mortalities

At site 284petkrfs (Figure 29), the water level and mosquito count followed a similar trend. An increase in mosquito count resulted in an increase in water level and a decrease in water level resulted in a decrease in mosquito count.

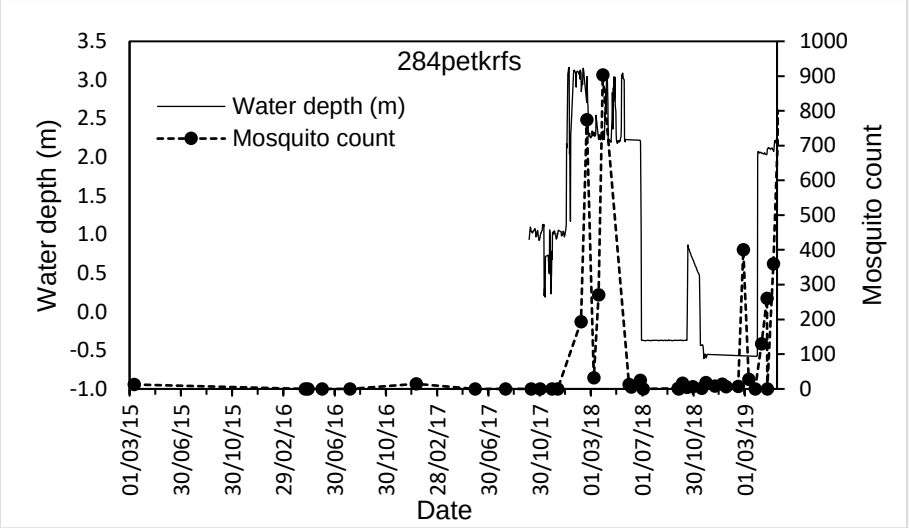


Figure 29 Relationship between water level depth and mosquito count in 284petkrfs.

For site 140troklkn (Figure 30), a high mosquito count was observed in March 2017 (387) following high amounts of rainfall. The water level, however, fluctuated between the years.

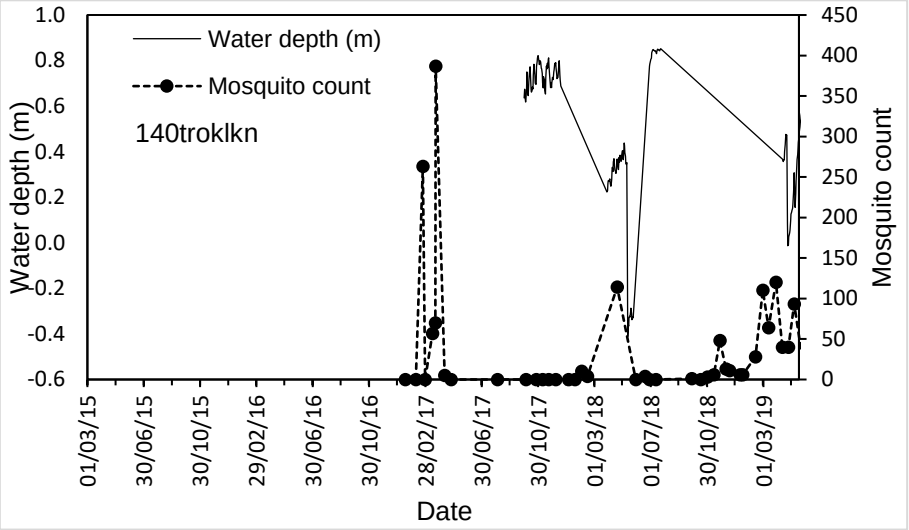


Figure 30 Relationship between water level depth and mosquito count in 140troklkn.

In 321hoppplr (Figure 31), the water level ranged between 0.9 and 1.4 m. In this site, very few mosquitoes were collected.

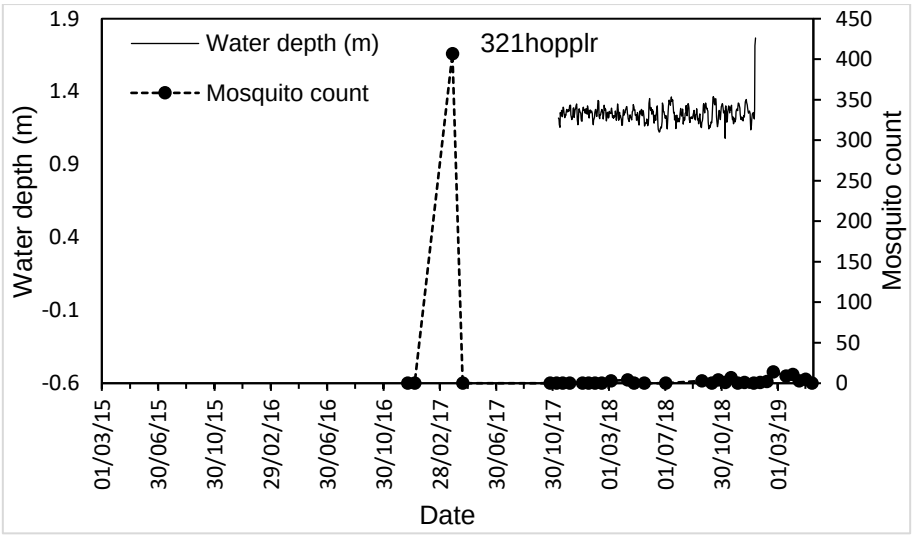


Figure 31 Relationship between water level depth and mosquito count in 321hoppplr.

At site 131smibssv (Figure 32), between 2017 and the beginning of February 2018 the mosquito count was zero. A drastic increase in mosquito count was observed from late February 2018 and it reached a peak of 2000 mosquito in March 2018. The water level during this period was fluctuating between 0 and 0.4 m. Towards the end of May 2018 to May 2019, a very small number of mosquitoes were collected.

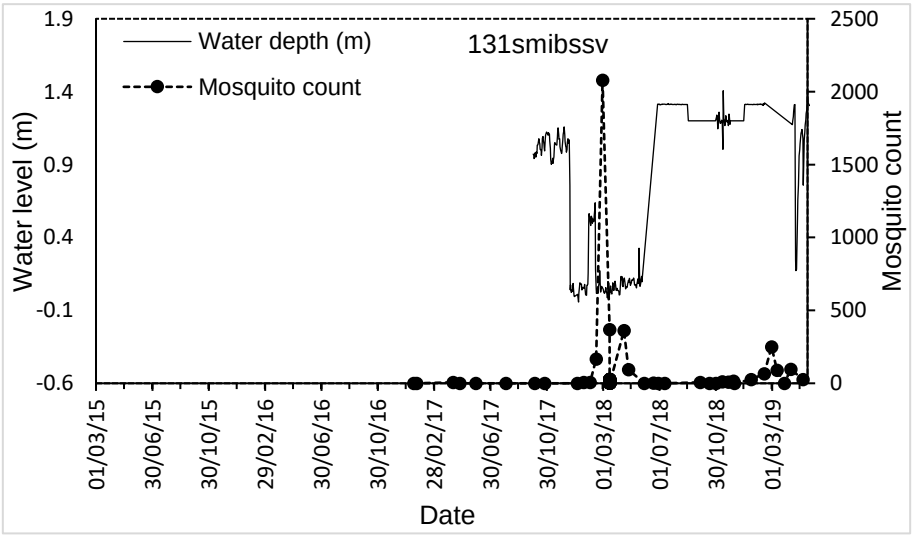


Figure 32 Relationship between water level depth and mosquito count in 131smibssv.

6.2.2.2 EM 50 sensors

The data from the EM 50 sensors suggested that the moisture sensors displayed irregular patterns from all four monitored depths. Sensor 1 (0-50 mm) typically showed an immediate response to the changes in water level because the soil surface was more subjected to climatic changes (rainfall, temperature, and evapotranspiration), groundwater level and soil texture also played a role. This pattern/ behaviour was observed in all sites monitored.

In p004bullmrl (Figure 33), the soil moisture content ranging between 0.1 and 0.3 (m^3/m^3) was observed in all depths. In months that received more rainfall (January-May), the moisture content was high, whereas, in months that received less rainfall, the moisture content was low. A similar situation was observed in p003bulmgkr and p002bulwltv. The sensor at depth one (50 mm) and depth two (100 mm), recorded lower moisture content when compared to the 3rd depth (350 mm). This might have been due to loamy sand texture in the first two horizons where water drained rapidly. The infiltration rate at the third depth might have been reduced because the texture changed to sandy clay loam. The mosquito count, on the other hand, showed a good relationship with soil moisture content. The mosquito count was 0 from 2016. There was, however, an increase of 230 mosquitoes in April 2017 with an increase in soil moisture content. Towards the end of May 2017, the mosquito count was back at 0 and the moisture content was decreasing. Another increase in mosquito count was observed at the beginning of 2018 with the mosquito count of 107 in April 2018. A maximum of 900 mosquitoes was collected in May 2019.

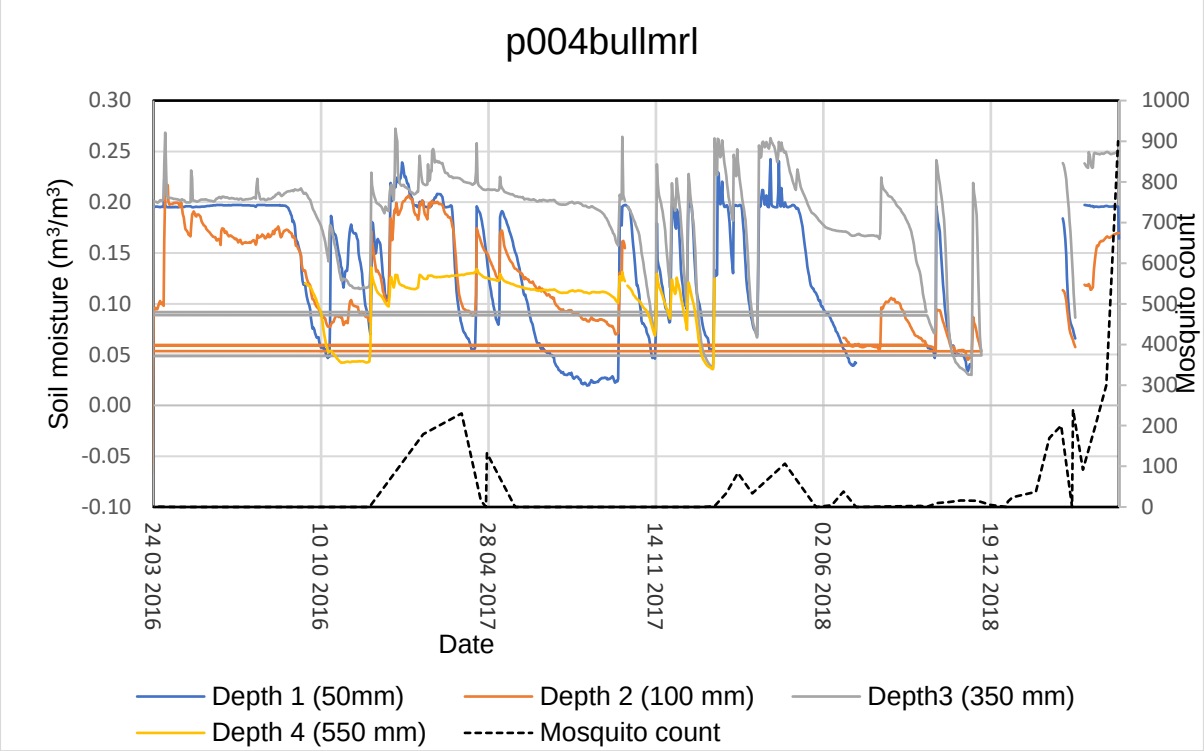


Figure 33 Relationship between soil moisture content and mosquito count in p004bullmrl.

In p003bulmgkr (Figure 34), soil moisture content ranged between 0 and 0.15 m^3/m^3. This site was characterised by the same soil texture (sandy clay loam) throughout the profile. The first depth displayed high soil moisture when compared to other depths. As depicted from the graph, it can be noted that with an increase in soil moisture there is an increase in mosquito abundance. In August 2018, an increase in soil moisture content was observed whereas the mosquito count was only 4.

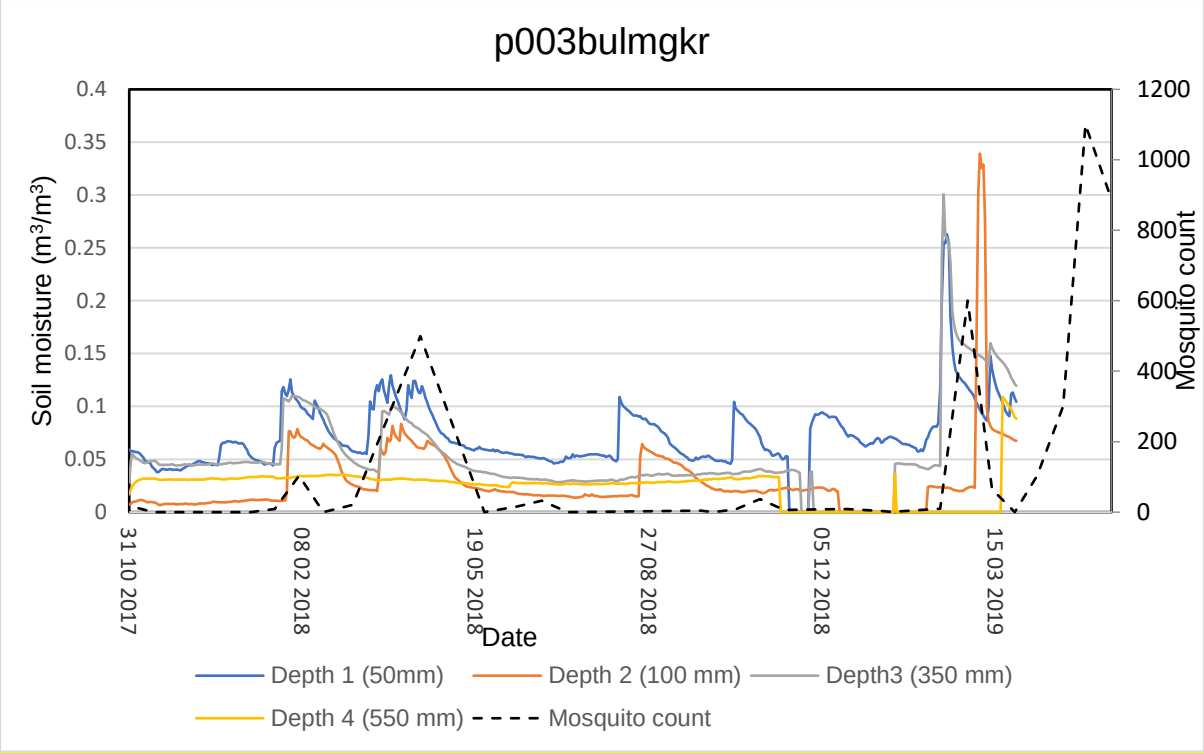


Figure 34 Relationship between soil moisture content and mosquito count in p003bulmgkr.

In p002bulwltv (Figure 35), there was a lot of missing data due to malfunctioning and damaged sensors. There was, however, an observed increase in mosquito count with an increase in soil moisture content in 2018 (February-April) and in April 2019.

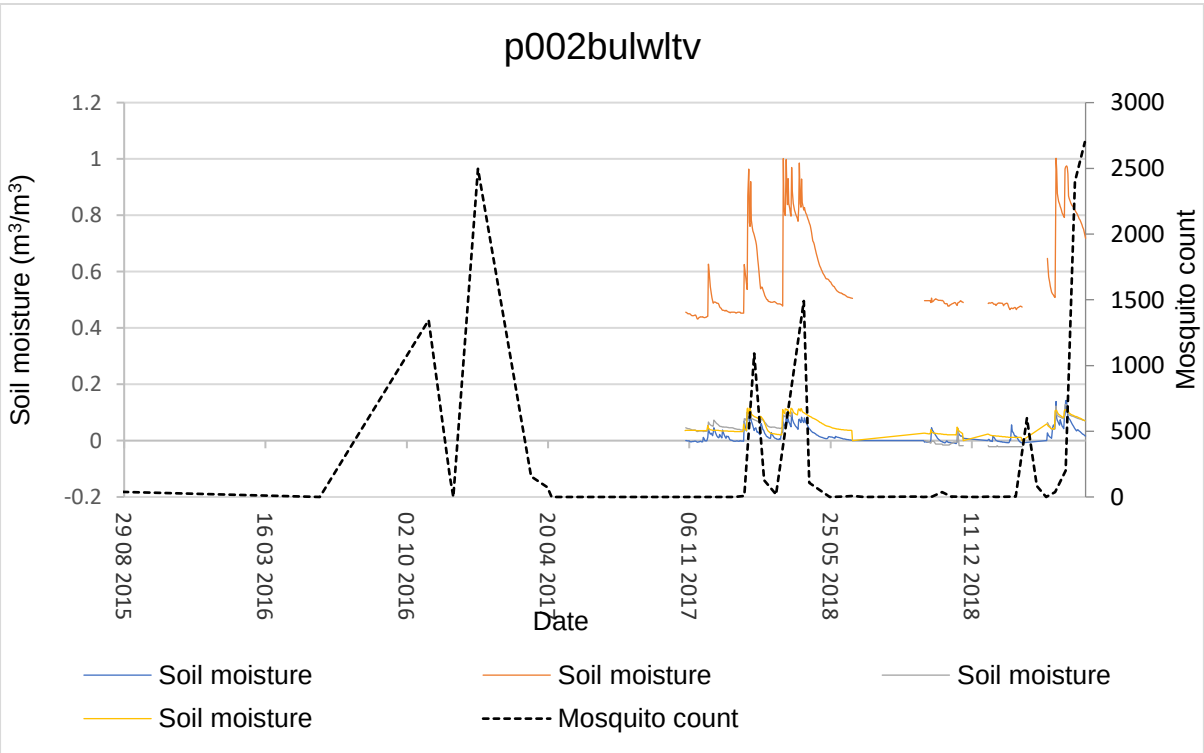


Figure 35 Relationship between soil moisture content and mosquito count in p002bulwltv.

6.2.2.3 Watermark sensors

All of the watermark sensors displayed a similar trend at all sites. Seasonal variations in the soil moisture content between the rainy and dry seasons were noted. However, during the rainy season, between January and May, in which adequate rainfall was expected, the moisture content, as well as the mosquito count, increased. This is likely because the sites have wetland soils that tend to be saturated for longer periods after rainfall and thus remain saturated even in the winter months. The rate of drying and the total amount of drying of wetlands differed between sites. During the drying period of the winter months, the mosquito count was less.

A fluctuation between 0-200 cbar from July 2017 to February 2018 was noticed. From February to June 2018, the soil was saturated and the moisture level ranged between 0-30 cbar. A gradual decrease was, therefore, noticed from July to November 2018 followed by a fluctuation. In March 2019 until June 2019, saturation in the soil was observed. Conversely, mosquito count showed an increase of 2080 in February 2018. A small increase in mosquito count was observed again in April 2018 and thereafter there was a mosquito count of less than 15. A small fluctuation was observed in 2019 as shown in Figure 36.

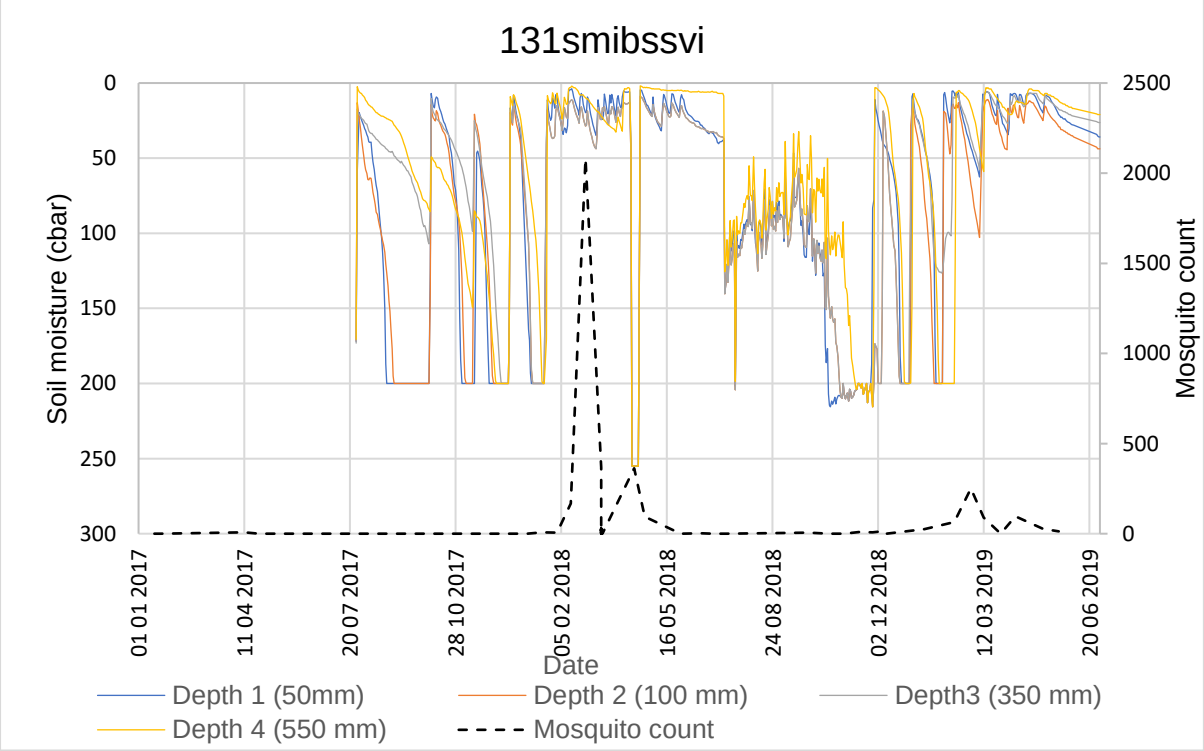


Figure 36 Relationship between soil moisture content and mosquito count in 131smibssv.

A great fluctuation (0-200 cbar of sensors between the four depths was observed throughout the years. The mosquito count was the highest in 2017 and from there the count was below 20 as shown in Figure 37.

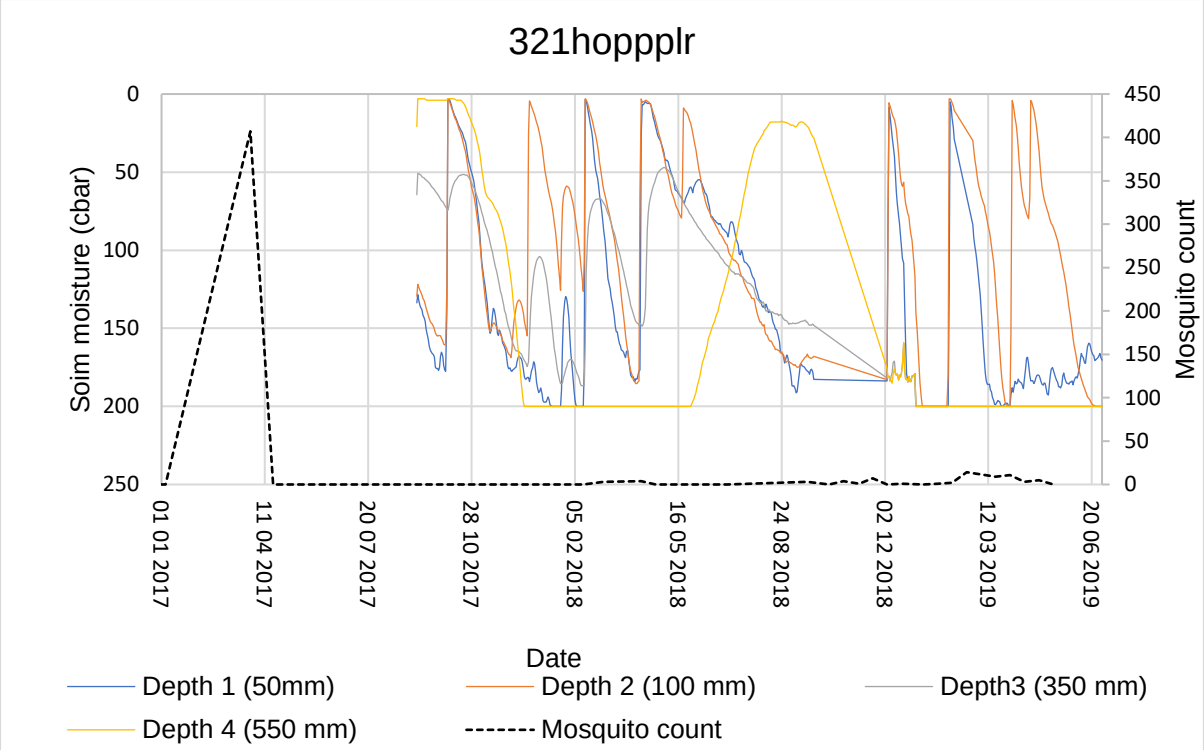


Figure 37 Relationship between soil moisture content and mosquito count in 321hoppplr.

The soil moisture levels initially ranged between 200 and 0 cbar. From December 2017, all the sensors remained saturated. A drastic decrease in sensor 3 was observed from July 2018 and thereafter showed a fluctuation between 250 and 56 cbar which shows that the sensor was dry. Mosquito count, on the other hand, was initially high and reached a count of 387 in March 2017. A low count was observed in April 2017. In April 2018, a count of 114 was achieved. A fluctuating mosquito count was seen from November 2018 and throughout 2019 as shown in Figure 38.

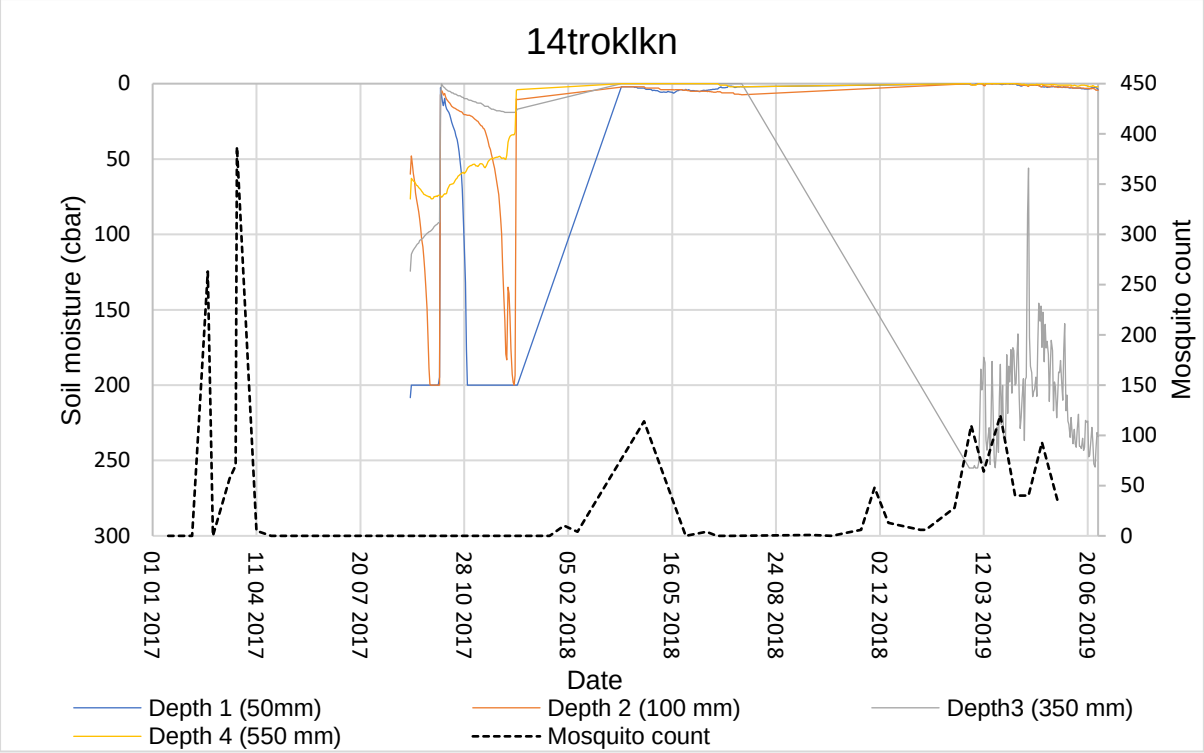


Figure 38 Relationship between soil moisture content and mosquito count in 140troklkn.

The watermark sensors resembled the same pattern in all the four depths. The sensors were initially dry (250-200 cbar) and there was a drastic increase to 0 cbar and back again at 200 cbar. From January 2018, the sensors were getting wet and approaching 0 cbar. From April 2018 to June 2019, the site was considerably wet. The mosquito count was initially low from January 2017, increased from February 2018, fluctuated throughout, until it reached a peak of 364 in April 2018. Thereafter, a decrease was observed until it reached 0 in May 2018. A gradual increase in mosquito count was observed from October 2018 and reached 300 in March 2019 and thereafter fluctuated throughout the months as shown (0-200 cbar) in Figure 39.

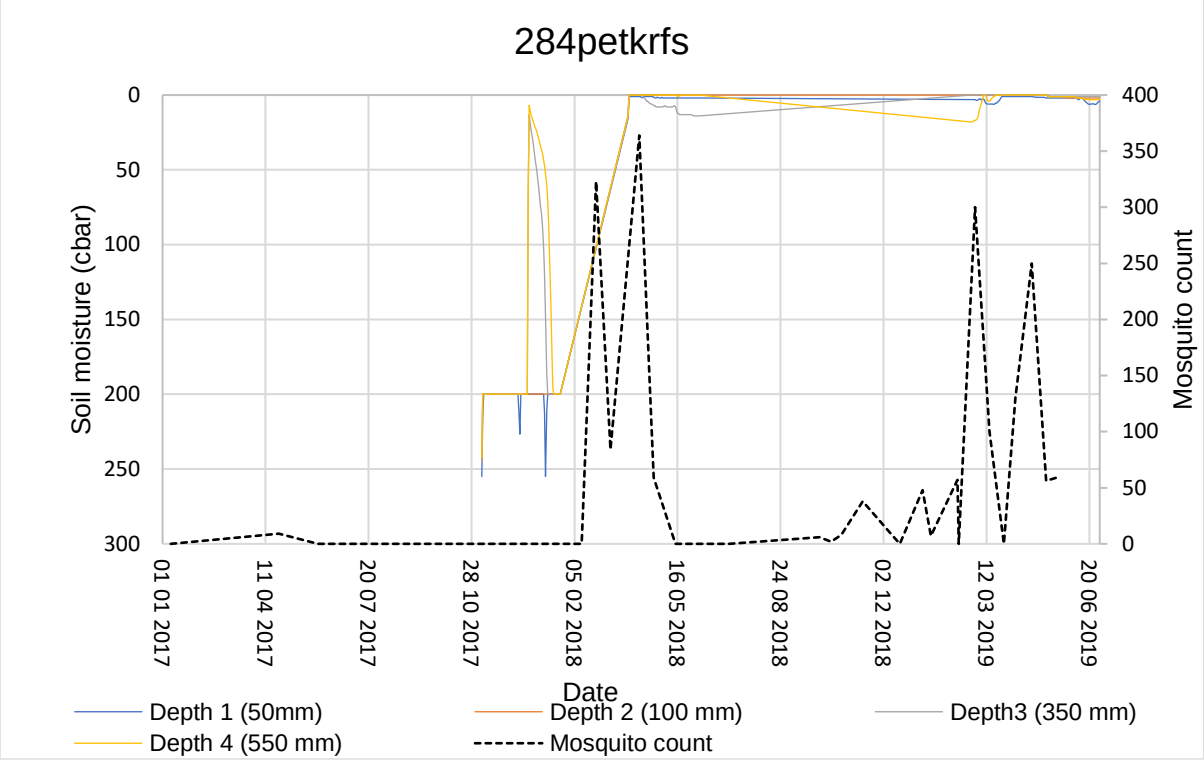


Figure 39 Relationship between soil moisture content and mosquito count in 284petkrfs.

All the water sensors were fluctuating between 0 and 200 cbar from October 2017 and in December 2017, all sensors gradually became saturated and approached 0 cbar. In March 2018, all the sensors were saturated and ranged between 0 and 39 cbar. From October 2018, sensors were getting dry and reached 200 cbar in November 2018. Sensors were saturated again from February 2019. In June 2019, the soil was getting dry. The mosquito count was initially high and decreased to zero. A count of 300 was achieved at the beginning of April 2019 as shown in Figure 40.

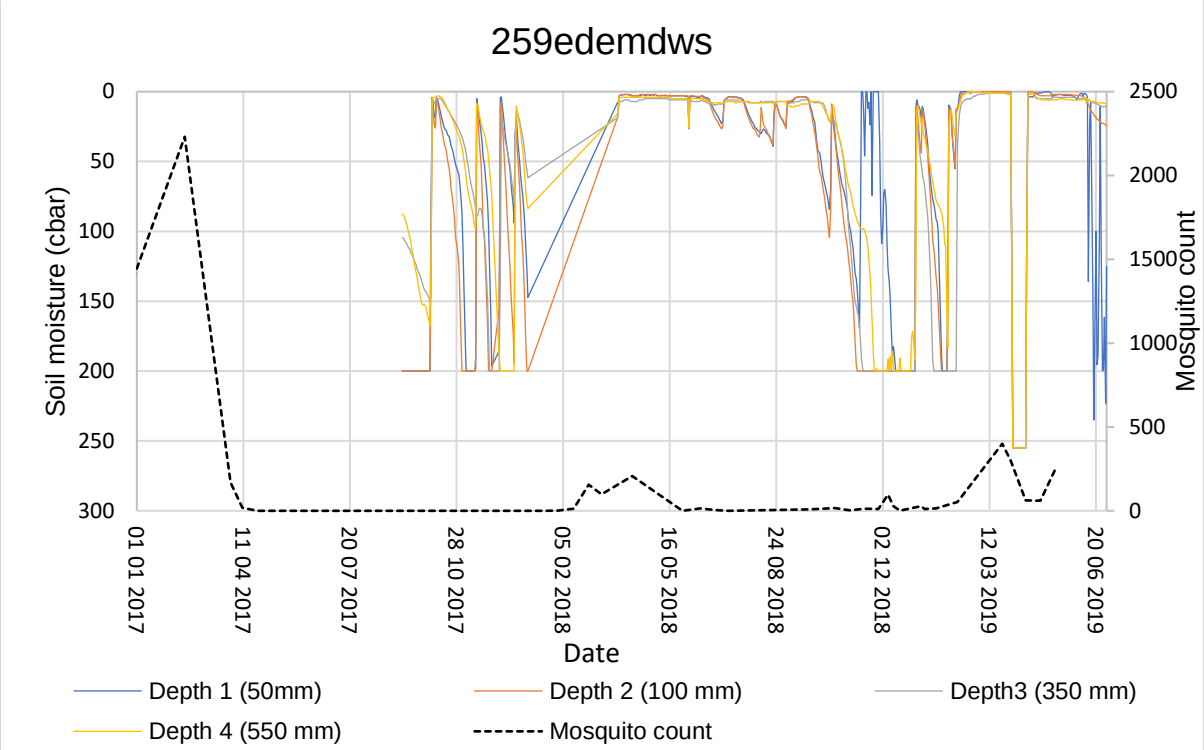


Figure 40 Relationship between soil moisture content and mosquito count in 259edemdws.

6.3 Discussion

The samples for microbial activity were analysed using the FDA method. All of the samples only included the topsoil. The study of Ladd *et al.* (1986) revealed that the soil surface has the highest microbial activity when compared to the subsoil. This is because the topsoil is rich in nutrients, is exposed to great amounts of energy inputs, and is highly influenced by environmental changes, including soil water and temperature (Ladd *et al.* 1986; Kaiser & Heinemeyer, 1993).

Microbial activity varied widely between the two years of sampling, although there was no clear trend. Some sites had the highest microbial activity in 2015 and the lowest in 2018, and for other sites, it was *vice versa*. The inconsistency in microbial activity between these two years might have been due to various reasons that include seasonal and annual variations with different wetting and drying periods and variations in soil temperature. Generally, in 2015-2016 South Africa underwent a drought period (Baudoin *et al*., 2017). Therefore, the sites received less rain, evapotranspiration rates were high, which all resulted in soil desiccation and thus possibly caused a decline in microbial activity in the soil in some sites. On the other hand, 2018 was considerably wetter, although the microbial activity was low for some sites. This might be further elucidated by comparing the methodology of Verster (2016) and the one followed in this study. In 2015, one sample was collected from one soil surface, while in 2018 soil was collected from six different locations on the soil's surface. The six samples were combined, mixed and a subsample was taken for analysis. Microbial activity can vary

considerably in soils, even if soil samples are taken very close to each other and, therefore, each sample collected contains a unique microbial activity at a specific location and time. Another reason that might explain the discrepancies between the results of 2015 and 2018, is that the samples for 2018 were collected during a rainy period and thus sometimes under waterlogged conditions. Waterlogged conditions decrease soil aeration and can cause a decline in soil microbial activity (Kaiser & Heinemeyer, 1993). Manipulation of the ecosystem by animals (cattle, sheep, buffaloes, rodents, insects, etc.) as a result of perturbation and natural ecosystem change over time, might have also influenced the microbes. Other biological, physical, and chemical properties might also have played a role (Bath & Soderstrom, 1982).

RVFV is believed to survive in transovarially infected *Aedes* mosquito eggs and it was previously mentioned that for these eggs to hatch, they require long periods of desiccation and 90% hatch during the initial flooding of the season (Logan *et al*., 1991). However, not all eggs hatch in response to the reasons mentioned above. Another factor that is considered to trigger hatching of dormant *Aedes* mosquito eggs is a reduction of dissolved oxygen concentration in the soil due to biological, chemical, and environmental factors (Gjullin *et al*., 1941; Ponnusamy *et al*., 2011). Gjullin *et al*. (1941) and Gillett *et al*. (1977), state that the presence of microorganisms in the water that the eggs become submerged in, results in a reduction in dissolved oxygen that therefore stimulates the hatching of eggs. Subsequently, Rozeboom (1934) and Gjullin *et al*. (1941), report that bacteria on their own have a stimulating effect on the hatching of the mosquito eggs. Moreover, Gillett *et al*. (1977) state that after oviposition, eggs secure bacteria/microbes colonies from the environment and thus lower the dissolved oxygen in the microenvironment of the eggs, which further stimulates the hatching of eggs. Bacteria required to influence the hatching of these eggs vary from egg to egg, that is the higher the bacteria count, the quicker the eggs will hatch. Also, eggs that are grouped have higher chances of hatching quicker as compared to eggs deposited singly and far apart from each other. *Aedes* mosquitoes lay eggs singly and thus the chances of these eggs hatching quicker are not as high compared to when they are grouped. This can, therefore, result in *Aedes* mosquitoes delaying hatching which in turn might contribute to outbreaks occurring after a long time.

Furthermore, it was found that the newly hatched mosquito larvae continue to survive near the eggs and feed on the soil surface. They thus reduce the bacterial growth around the eggs, decreasing the number of bacteria per egg and therefore delay egg hatching (Gillett *et al*., 1977). Although mosquito eggs were not collected in this study, it is possible that the microbial activity, bacteria, in particular, played a role in the hatching of *Aedes* mosquito eggs.

A population of mosquitoes in different genus fluctuated during the study period. More than 378 811 mosquitoes belonging to five different genera were identified in the study sites, of which *Aedes* dominated followed by *Culex*. This is because most *Aedes* mosquitoes hatch during the first flooding, whereas *Culex* requires standing water to lay eggs. These two mosquitoes' species are often associated with arbovirus transmission, including the Rift Valley fever virus. *Anopheles* species on the other hand were found in low numbers.

Climate can positively or negatively influence the vector population size, of which rainfall, temperature, and humidity are the most important variables. These variables can, therefore, be used to identify the periods when the vectors thrive the most and when a peak in virus transmission can be expected. In particular, RVFV is influenced by above-average rainfall, which results in high soil water levels and promotes flooding. Upon flooding, hatching of mosquito eggs is encouraged and a good environment for larvae development is created. This in turn influences mosquito population growth and thus increases the potential of RVF outbreaks. Low temperatures and high humidity also create a conducive environment for mosquito population growth. In the current study, a relationship between rainfall, soil water levels, and the abundance of mosquitoes were observed in the study sites where high vector populations were detected in months with above normal rainfalls. There are months where high rainfall and flooding were observed, whereas the mosquito abundance is low or decreased. This might be due to various reasons that include more *Aedes* mosquitoes' eggs hatch during the first flooding than during subsequent flooding (Logan *et al.,* 1991). This was observed in the following sites: p010jacrtrv and p003bulmgkrthe where eggs hatch after the first inundation, with some eggs only hatching after repeated inundation. Mosquito larvae could also have been washed off before they are fully developed due to above normal rainfall and thus it would decrease mosquito abundance.

In contrast, during winter months hatching of mosquitoes is low. This is because there is less rainfall received and it is a cold season and therefore, less evapotranspiration and thus lower number of mosquito count. Also, during the drought period, there is less rainfall received and therefore, there is no conducive environment for the breeding of mosquitoes as it was observed in most sites in 2015 and 2016. Some sites have standing water during the drought period and winter months and this is due to wetlands that were saturated in summer months. In winter, the soil water content decreases gradually until it dries totally in late winter and early spring. Irrigation plays a role in saturating the wetlands and thus improving the mosquito count, as it was seen in site p010jacrtrv with 3415 mosquito count in winter and the drought period.

6.4 Conclusions

Changes in microbial activity in the soil are influenced by climate, particularly rainfall and temperature. These changes result in an increase or decrease in microbial growth and activity. Microbial activity is sensitive to extreme weather conditions. Although microbial activity plays a crucial role in the stimulating of eggs to hatch, however, it might not be a good indicator of the outbreaks of RVF. The results displayed no statistical significance of microbial activity between mortality sites and sites without mortalities in 2015 and 2018. *Aedes* mosquitoes dominate all the mosquitoes collected, however, hatching is limited after the first flooding because *Aedes* mosquito eggs require long period of desiccation. Subsequent flooding of the wetlands creates a conducive environment for the hatching of *A. culex* mosquito eggs because they require a longer period of freestanding water. Other factors that may limit hatching of mosquitoes include cold and windy conditions and the drought years. This may further elucidate the reason why the outbreaks of RVF occur after a long period.

Chapter 7: Validation of the discriminant function for predicting sites with RVF

7.1 Introduction

This chapter aimed to validate the existing discriminant function based on soil properties for predicting sites with RVF-associated mortalities. A previous study by Verster (2016) was conducted across 22 sites, which was expanded with eight (8) new sites for the present study. All these sites were classified according to the World Organisation for Animal Health (OIE) 2010 report, as either sites with or without RVF associated mortalities. According to this classification, 12 of the 22 sites were sites with mortalities and the remaining ten (10) were sites without mortalities. All 8 new sites were classified as sites where mortalities were not reported.

7.2 Results

The linear discriminant function to discriminate between sites with and without reported RVF mortality was developed by Verster *et al.* (2020) based on the original 22 sites. This discriminant function was subsequently applied to classify the 8 additional sites to evaluate the existing discriminant function proposed by Verster *et al.* (2020). The discriminant function subsequently classified seven (7) new additional sites as sites with RVF-associated mortalities (Table 17). 211bosxcls was the only site that was classified as a site without mortality.

The following linear discriminant function was used to predict if a site had or did not have reported RVF mortalities:

$$D_g\ (x_i) = x_i' L_g + C_g$$

Here, for a given wetland *i*, x_i is the vector of four dimensions containing the values of the variables: sqrt (exchangeable K^+), ln (exchangeable Mg^{2+}), sqrt (medium sand), and ln (Br). The vectors, L_g and constants C_g were as follows (outputs of the SAS procedure "DISCRIM"), where g = group:

$$L_1 = \begin{pmatrix} 29.828 \\ 14.982 \\ 7.825 \\ 1.318 \end{pmatrix}$$
$$C_1 = -42.773$$
$$L_2 = \begin{pmatrix} 38.720 \\ 19.937 \\ 11.943 \\ -0.225 \end{pmatrix}$$
$$C_2 = -71.929$$

For each location *i*, the distances $D_g\ (x_i)$ of x_i from the two groups, g = 1, 2 are calculated and then location *i* is allocated to Group 1 if $D_1\ (x_i) < D_2\ (x_i)$ otherwise, if $D_1\ (x_i) > D_2\ (x_i)$, the location *i* is allocated to Group 2.

Table 17 Posterior probability classification of membership to sites with and without RVF associated mortalities, using the linear discriminant function proposed by Verster *et al.* (2020)

	Mortality classification		Membership probability	
Sites name	OIE report[1]	Discriminant function[2]	Site with mortalities	Site without mortalities
321hoppplr	without mortalities	with mortalities	0.76	0.24
222phibthl	without mortalities	with mortalities	0.93	0.07
259edemdws	without mortalities	with mortalities	0.59	0.40
211bosxcls	without mortalities	without mortalities	0.22	0.77
284petkrfs	without mortalities	with mortalities	0.98	0.02
140troklkn	without mortalities	with mortalities	0.99	0.00
131smibssv#1	without mortalities	with mortalities	0.99	0.00
131smibssv#2	without mortalities	with mortalities	0.99	0.00

[1] OIE (2010)
[2] From Verster *et al.* (2020)

7.3 Discussion

The misclassification of the sites could have been due to differences in soil properties. At all sites, in the soil properties that were tested, the following relationships were found: CEC, cations, and clay content depicted a good relationship between the new sites without mortalities and sites with mortalities. Conversely, the relationship with the old sites without reported mortalities was weak. Organic carbon was the only soil property that showed a good relationship between old sites without mortalities and new sites without mortalities. Other soil properties (i.e. phosphorus with the Olsen, $CaCl_2$, and H_2O extraction), pH (H_2O and KCl), nitrogen, sand (medium, fine, and very fine) and coarse silt) showed a good relationship in all sites. The soil classification also showed a good relationship between mortality sites and new sites without mortalities.

The original discriminant function by Verster *et al.* (2020) was developed as follows: Van der Waerden test was used to select each soil properties that were either near significant or significantly different between sites with and without mortalities. For each of the first three soil layers, the Box-Cox method was used to transform data to normality. This resulted in the determination of the power transformation. The best overall power transformation across the layers was subsequently chosen through inspection (this was grounded on likelihood profiles as a function of the power parameter). The average of the first three layers (after applying the selected power transformation to normality), in each site and for every transformed variable, was used. This excluded the microbiological and the mineralogical variables since only one layer was assayed. The SAS procedure was used for a stepwise selection of the variables to

be included in the discriminant function from the averaged and transformed variables. For variables to be selected for the discriminant function, a 0.1 significance level was used. The selected variables led to quadratic discriminant analysis. Cross-validation was subsequently used to estimate the misclassification rate of the quadratic discriminant function.

7.4 Conclusions

The existing discriminant function coined by Verster *et al.* (2020), did not work well when applied to the 8 new sites. Only one of the eight sites were correctly classified. In future studies, a larger sample size, i.e. more sites should be considered. It should also very carefully be considered how mortalities sites and sites without mortalities are defined and determined as opposed to relying on the OIE report.

Chapter 8: Conclusions and recommendations

8.1 Conclusions

The One Health concept aims to promote that people do not exist independently but are part of the ecosystem where the action of one member affects other members. In this study, the One Health concept and principles are followed which aim to bring various scientific disciplines (people, animals, and the ecosystem) at a local, national and global level to improve health for all. This research formed part of a broader study, aiming to understand the occurrence and prevalence of Rift Valley fever in South Africa. The broader study involves a variety of personnel such as climatologists, vegetation specialists, epidemiologists, veterinarians, entomologists, anthropologists, and soil scientists. An initial attempt to study the relationship of RVF with soil was conducted by Verster (2016) and concluded by using soil properties to predict sites that are susceptible to RVF outbreaks. This study was built on the study of Verster (2016), through improved understanding between soil research and other relevant disciplines that included mosquitoes, Rift Valley fever virus, vegetation, and climate.

Through statistical analysis, the following soil properties were identified to be potential predictors of RVF mortalities:

1. Chemical analyses: Soil chemical properties with significant relationships were CEC only in the 4th layer, exchangeable Ca^{2+} in all horizons, exchangeable K^{+} only in the A horizon, and B1 horizon and exchangeable Mg^{2+} in the first three horizons. Exchangeable Na^{+} had no significant relationship. For soluble cations, soluble Ca^{2+} and K^{+} were the only cations with significant relationships. The values were all lower in sites with mortalities than in sites without mortalities. This was similar to the findings of Verster (2016).
2. Physical analyses: Only coarse silt in the A horizon displayed a significant difference between sites with mortalities and sites without mortalities. It was higher in sites without mortalities than in sites with mortalities. Coarse silt has a lower water holding capacity than clay and can thus result in drier environments that do not favour mosquito eggs. In the study by Verster (2016), the medium sand was found to be significantly different from the mean being lower in sites with RVF mortalities than sites without mortalities. However, Brand *et a*l. (2018) did a visual field assessment of soil types and observed that clay is the more common soil texture in sites with RVF mortalities due to the ability to hold more water for longer periods.
3. Soil classification: Kastanozems, Calcisols, Vertisols, Mollic Stagnosols, and Mollisols dominated sites with RVF mortalities and the new sites without RVF mortalities. These

sites were also characterised by the accumulation of carbonates, high base saturation, and stagnic properties which all indicate soils that can retain water for longer as compared to the old sites without RVF mortalities.

4. Mineralogical analyses: Significant differences between sites with RVF mortalities and sites without RVF mortalities were only observed in elements such as Ba, Ni, Y, and Zn. The Ni, Y, and Zn were higher in sites without RVF mortalities and might have suppressed the RVF virus mosquito eggs in the soil. Ba was higher in RVF mortality sites.
5. There was no significant difference in microbial activity between sites with RVF mortalities and sites without RVF mortalities and between the two different years of sampling. The microbial activity varied widely in the soil where some sites had high microbial activity in 2015 and low microbial activity in 2018, and *vice versa*. The lack of differences was possibly due to different soil sample collection localities and differences in climate, 2018 was quite moist, while 2015 was dry. It was also observed that microbial activity was sensitive to extreme weather conditions such as drought periods and waterlogged areas.
6. Apart from *Aedes* mosquito eggs hatching from repeated wetting cycles, they can also hatch through the presence of microorganisms in the soil resulting in dissolved oxygen and thus trigger hatching of eggs (Gjullin *et al*., 1941; Gillett *et al*., 1976).
7. Monitoring of wetlands: The on-going monitoring of soil water content in wetlands is essential because high water content results in flooding and thus creates a suitable environment for a high population of mosquito vectors which favour breeding and successive outbreaks (Linthicum *et al*., 2016; Mlingano Agricultural Research Institute, 2006).
8. This relationship between the soil water content and mosquito population was observed in the study where with high soil moisture levels the mosquito abundance increased. This relationship was likely to occur between summer rainfalls to early autumn (Jan-April). Certain soils can remain water-saturated even after these rainfall months, due to the low-lying position in the landscape, the higher clay content and the clay mineralogy. There are thus months where the soil moisture levels were high, but the mosquito population was low due to 90% of *Aedes* mosquito eggs hatching during the first flooding. In winter months, there was less rainfall and it was colder, therefore, mosquitoes' activity is suppressed. In countries that experience warm winters, the mosquito activity can continue. Also, in August, during windy days the mosquito count was less. This data should be explored further to identify and monitor areas of high mosquito prevalence.

9. The new sites were finally classified, using the procedure of Verster (2016), as either sites with RVF mortality or sites without RVF mortality. All the new sites were thus classified as sites without RVF mortalities. The discriminant function of Verster (2016), therefore, misclassified seven of the new sites as sites with RVF mortalities, while only one site was classified correctly as a site without RVF mortalities.

The emergence and re-emergence of especially viral infectious diseases are becoming a global concern and threat, such as Covid-19 serving as a current example. Based on the data presented here, the geographic probability of RVF outbreaks can be predicted using soil parameters. These areas can then be monitored for soil water content, mosquito data, and climate to predict the probability of an RVF outbreak.

8.2 Recommendations

1. Soil microbial activity should be taken annually and at least four times per year, in summer, autumn, winter, and spring.
2. The soil water sensors should be fenced off to avoid being vandalised by animals, which resulted in data loss for this study.
3. *Aedes* mosquito eggs should be collected and tested from sites with RVF mortalities and sites without RVF mortalities.
4. The study of wetland soil properties should be expanded to other provinces (KwaZulu-Natal, Limpopo and Eastern Cape) where RVF outbreaks have occurred to compare the differences and similarities in soil properties between the different climate regions and between sites with RVF mortalities and sites without RVF mortalities.
5. The geology and lithology under different climate and environmental conditions should also be considered to determine how it influences the soils' properties.

Chapter 9: References

ADAM, G. AND DUNCAN, H., 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry*, 33, 943-951.

AMORE, D.V.D., STEWART, S.R. AND HUDDLESTON, J.H., 2004. Saturation, reduction, and the formation of iron-manganese concretions in the Jackson-Frazier wetland, Oregon. *Soil Science Society of America Journal,* 68,1012-1022.

ANYAMBA, A., CHRETIEN, J.P., SMALL, J., TUCKER, C.J., FORMENTY, P.B., RICHARDSON, J.H., BRITCH, S.C., SCHNABEL, D.C., ERICKSON, R.L. AND LINTHICUM, K.J., 2009. Prediction of a Rift Valley fever outbreak. *Proceedings of the National Academy of Sciences,* 106, 955-959.

ANYAMBA, A., LINTHICUM, K.J., BRITCH, S.C., AND TUCKER, C.J., 2001. Climate-diseases connection: Rift Valley fever in Kenya. *Cadernos de saude publica*, 17, 133-140.

ANYAMBA, A., LINTHICUM, K.J., SMALL, J., BRITCH S.C., AND TUCKER, C.J., 2012. Remote sensing contribution to prediction and risk assessment of natural disasters caused by large scale Rift Valley fever outbreaks. *Proceedings of the IEEE*, 100, 2824-2834.

ANYAMBA, A., LINTHICUM, K.J., SMALL, J., BRITCH, S.C., PAK, E., DE LA ROCQUE, S., FORMENTY, P., HIGHTOWER, A.W., BREIMAN, R.F., CHRETIEN, J.P., TUCKER, C.J., SCHNABEL, D., SANG, R., HAAGSMA, K., LATHAM, M., LEWANDOWSKI, H.B., MAGDI, S.O., MOHAMED, M.A., NGUKU, P.M., REYNES, J. AND SWANEPOEL, R., 2010. Prediction, assessment of the Rift Valley fever activity in East and Southern Africa 2006-2008 and possible vectors control strategies. *American Journal of Tropical Medicine and Hygiene*, 83, 43-51.

Anonymous, 2014, https://web.stanford.edu/group/glam/xlab/MatSci162_172/LectureNotes/07_Geometry,%20Detectors.pdf (Accessed 05/09/2018)

ARADAIB, I.E., ERICKSON, B.R., ELAGEB, R.M., KHRISTOVA, M.L., CARROLL, S.A., ELKHIDIR, I.M., KARSANY, M.E., KARRAR, A.E., ELBASHIR, M.I. AND NICHOL, S.T., 2013. Rift Valley fever, Sudan, 2007 and 2010. *Emerging infectious diseases*, 19, 246.

ARCHER, B.N., THOMAS, J., WEYER, J., CENGIMBO, A., LANDOH, D.E., JACOBS, C., NTULI, S., MODISE, M., MATHONSI, M., MASHISHI, M.S. AND LEMAN, P.A., 2013. Epidemiologic investigations into outbreaks of Rift Valley fever in humans, South Africa, 2008–2011. *Emerging Infectious Diseases*, 19, 1918-1925.

ARCHER, B.N., WEYER, J., PAWESKA, J., NKOSI, D., LEMAN, P., TINT, K.S. AND BLUMBERG, L., 2011. Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. *South African Medical Journal*, 101, 263-266.

ATSDR, U., 2007. Toxicological profile for lead. *US Department of Health and Human Services*, 1, 582.

BAATH, E. AND SODERSTRÖM, B., 1982. Seasonal and spatial variation in fungal biomass in a forest soil. *Soil Biology and Biochemistry*, 14, 353-358.

BABA, M., MASIGA, D.K., SANG, R. AND VILLINGER, J., 2016. Has Rift Valley fever virus evolved with increasing severity in human populations in East Africa? *Emerging microbes and infections*, 5, 1-10.

BALKHY, H.H. AND MEMISH, Z.A., 2003. Rift Valley fever: an uninvited zoonosis in the Arabian Peninsula. *International Journal of Antimicrobial Agents*, 21, 153-157.

BANSAL, O.P., 2018. The influence of potentially toxic elements on soil biological and chemical properties. In *metals in soil-contamination and remediation*. IntechOpen.

BAUDOIN, M.A., VOGEL, C., NORTJE, K. AND NAIK, M., 2017. Living with drought in South Africa: lessons learnt from the recent El Nino drought period. *International Journal of Disaster Risk Reduction*, 23, 128-137.

BISHOP, D.H.L., CALISHER, C.H., CASALS, J., CHUMAKOV, M.P., GAIDAMOVICH, S.Y., HANNOUN, C., LVOV, D.K., MARSHALL, I.D., OKER-BLOM, N., PETTERSSON, R.F. AND PORTERFIELD, J.S., 1980. Bunyaviridae. *Intervirology*, 14,125-143.

BORTOLOTTI, M., LUTTEROTTI, L. AND PEPPONI, G., 2017. Combining XRD and XRF analysis in one Rietveld-like fitting. *Powder Diffraction*, 32, 225-230.

BOWELL, R.J., ALPERS, C.N., JAMIESON, H.E., NORDSTROM, D.K. AND MAJZLAN, J., 2014. The Environmental Geochemistry of arsenic an overview. *Reviews in Mineralogy and Geochemistry*, 79, 1-16.

BRAACK, A.M., WALTERS, D. AND KOTZE, D.C., 2000. Practical wetland management. *South Africa Rennies wetland project.*

BRAND, R.F., ROSTAL, M.K., KEMP, A., ANYAMBA, A., ZWIEGERS, H., VAN HUYSSTEEN, C.W., KARESH, W.B. AND PAWESKA, J.T., 2018. A phytosociological analysis and description of wetland vegetation and ecological factors associated with locations of high mortality for the 2010-2011 Rift Valley fever outbreak in South Africa. *Public Library of Science one*, *13*.

BRANDER, L. AND SCHUYT, K., 2010. Benefits transfer: The economic value of world's wetlands. http://www.cbd.int/financial/values/g-value-wetlands.pdf (Accessed 05/09/2018)

BROWN, L.R. AND COOK, C., 2014. Wetlands delineation: Portion 531 of the farm Elandsfontein 108 IR and the remainder of portion 2 of the farm Elandsfontein 90 IR. Enviroguard Ecological Servces cc.

CAMPBELL, C.A. AND BIEDERBECK, V.O., 1976. Soil bacterial changes as affected by growing season weather conditions: a field and laboratory study. *Canadian Journal of Soil Science*, 56, 293-310.

CANADIAN COUNCIL OF MINISTERS OF THE ENVIRONMENT. 2013. Canadian soil quality guidelines for the protection of environmental and human health: Barium. *In:* Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, Winnipeg.

CHOUDHURY, H., CARY, R. AND WORLD HEALTH ORGANIZATION, 2001. Barium and barium compounds. World Health Organization.

Clark, T.M., Flis, B.J. and Remold, S.K., 2004. Differences in the effects of salinity on larval growth and developmental programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). *Journal of Experimental Biology*, *207*, 2289-2295.

COBOS, D.R. AND CHAMBERS, C., 2010. Calibrating ECH2O soil moisture sensors. *Application note,* 1-5.

COETZER, J.A., 1977. The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. *Onderstepoort Journal of Veterinary. Research*, 44, 205–212.

COUNCIL, GREATER WELLINGTON REGIONAL, 2005. Understanding the 'wet'in wetlands. A guide to the management of freshwater wetland hydrology. *Wellington: Greater Wellington Regional Council*.

COWARDIN, L.M., CARTER, V., GOLET, C. AND LAROE, E.T., 1979. Classification of wetlands and deepwater habitats of the United States. Fish and Wildlife Service, US Department of Interior. Washington, DC.DAUBNEY, R., HUDSON, J.R. AND GARNHAM, P.C., 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa. *The Journal of pathology and bacteriology*, 34, 545-579.

DAUTU, G., SINDATO, C., MWEENE, A.S., SAMUI, K.L., ROY, P., NOAD, R., 2012. Rift Valley fever: Real or perceived threat for Zambia, *Onderstepoort Journal of Veterinary Research*, 6, 79- 466

DAVIES, F.G., 1975. Observations on the epidemiology of Rift Valley fever in Kenya. *Epidemiology and Infection*. 75, 219-230.

DAVIES, F.G., 2010. The historical and recent impact of Rift Valley fever in Africa. *The American Journal of Tropical Medicine and Hygiene*, *83*, .73 -74.

DAVIES, F.G., KILELU, E., LINTHICUM, K.J. AND PEGRAM, R.G., 1992. Patterns of Rift Valley fever activity in Zambia. *Epidemiology and Infection*, 108, 185-191.

DAVIES, F.G., LINTHICUM, K.J. AND JAMES, A.D., 1985. Rainfall and epizootic Rift Valley fever. *Bulletin of the World Health Organisation,* 63, 941-943.

DRIESSEN, P., DECKERS, J., SPAARGAREN, O. AND NACHTERGAELE, F., 2001. Lecture notes on the major soils of the world, World soil resources reports 2000, 94, 334, ISSN 0532-0488. *Record Number 20023015720*.

DUGAN, P., 1990. Wetland conservation*: In:* P.J. Dugan (eds). A review of current issues and required action. IUCN. Gland, Switzerland.

DWAF (Department of Water Affairs and Forestry), 2005 A pratical procedure for identification and deliniation of wetlands and ripirian areas. Department of Water Affairs and Forestry, Pretoria.

EWART-SMITH, J.L., OLLIS, D.J., DAY, J.A. AND MALAN, H.L., 2006. National wetland inventory: Development of a wetland classification system for South Africa. WRC Report No. KV 174/06. Water Research Commission, Pretoria

FAO/WHO, 2008. Rift Valley fever outbreaks forecasting models. Rome, Italy. https://www.who.int/csr/resources/publications/WHO_HSE_GAR_BDP_2009_2/en/ (Accessed 05/06/2018)

FARRAH, S.R., BITTON, G.A.B.R.I.E.L., BOLLAG, J. AND STOTZKY, G., 1990. Viruses in the soil environment. *Soil biochemistry,* 6, 529-556.

FIEDLER, S, VEPRASKAS, M.J AND RICHARDSON, J.L., 2007. Soil Redox Potential: Importance, Field Measurements and Observations. *Advances in Agronomy*, 94, 1–54.

GEAR, J., DE MEILLON, B., MEASROCH, V. AND DAVIS, D.H.S., 1951. Rift Valley fever in South Africa: 2: the occurrence of human cases in the Orange Free State, the North-Western Cape Province, the Western and Southern Transvaal: B: field and laboratory investigations. *African Journal of Health Professions Education*, *25*, 908-912.

GERDES, G.H., 2004. Rift Valley fever. Scientific and Technical Review of the Office *International des Epizooties*, 23, 613-623.

GILLETT, J.D., ROMAN, E.A. AND PHILLIPS, V., 1977. Erratic hatching in *Aedes* eggs: A new interpretation. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 196, 223-232.

GJULLIN, C., HEGARTY, C. AND BOLLEN, W., 1941. The necessity of a low oxygen concentration for the hatching of *Aedes* mosquito eggs. *Journal of Cellular and Comparative Physiology*, 17, 2.

GF-TADs (Global framework for the progressive control of transboundary animal disease), 2012. Global framework for the progressive control of transboundary of animal disease. http://www.gf-tads.org/ (Accessed 05/09/2019)

GOUDIE, A.S. AND WELLS, G.L., 1995. The nature, distribution and formation of pans in arid zones. *Earth-Science Reviews*, *38*, 1-69.

HENNESSY, J.T., GIBBENS, R.P., TROMBLE, J.M. AND CARDENAS, M., 1983. Water properties of caliche. *Journal of Range Management*, 723-726.

HENSLEY, M., LE ROUX, P., DU PREEZ, C., VAN HUYSSTEEN, C., KOTZE, E. AND VAN RENSBURG, L., 2006. Soils: the Free State's agricultural base. *South African Geographical Journal*, 88, 11-21.

HIGHTOWER, A., KINKADE, C., NGUKU, P.M., ANYANGU, A., MUTONGA, D., OMOLO, J., NJENGA, M.K., FEIKIN, D.R., SCHNABEL, D., OMBOK, M. AND BREIMAN, R.F., 2012. Relationship of climate, geography and geology to the incidence of Rift Valley fever in Kenya during the 2006-2007 outbreak. *The American journal of tropical medicine and hygiene*. 86, 373-380.

HOLMES, P. AND BARKER, C.H., 2006. Geological and geomorphological controls on the physical landscape of the Free State. *South African Geographical Journal*, 88, 3-10.

HOLMES, P.J. AND BOARDMAN, J., 2018. *Southern African landscapes and environmental change*. Routledge.

HOLMES, W.E. AND ZAK, D.R., 1994. Soil microbial biomass dynamics and net nitrogen mineralization in northern hardwood ecosystems. *Soil Science Society of America Journal*, 58, 238-243.

HOSHEN, M.B. AND MORSE, A.P., 2004. A weather-driven model of malaria transmission. *Malaria journal*, 3, 32.

HURT, G.W. AND CARLISLE, V.W., 2001. Delineating hydric soils. *Wetland Soils: Genesis, Hydrology, Landscapes, and Classification,* 183-206.

IKEGAMI, T. AND MAKINO, S., 2011.The pathogenesis of Rift Valley fever. *Viruses*, 3, 493-519.

INGLETT, P.W., REDDY, K.R.,CORSTANJE.R, 2005. Anaerobic soils. *In:* J.L. Hatfield, K.M. Scow, D.S. Powlson, M.J. Singer, C. Rosenzweig and D.L Sparks (eds). Encyclopedia of Soils in the Environment. New York.

IPCC AR4 SYR. 2007. Core writing team: Pachauri RK, Reisinger A (eds). *Climate change 2007: Synthesis Report*. Contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC. ISBN 92-9169-122-4.

ISBELL, J.L., COLE, D.I. AND CATUNEANU, O., 2008. Carboniferous-Permian glaciation in the main Karoo Basin, South Africa: Stratigraphy, depositional controls, and glacial dynamics. In *Resolving the Late Paleozoic ice age in time and space*. *Geological Society of America Special Paper*, 441, 71-82.

IUSS WORKING GROUP WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106. FAO, Rome.

JENNY, H., 1994. Factors of soil formation: a system of quantitative pedology. Dover publications Inc, New York.

JOHNSON, M.R., VUUREN, C.J., VISSER, J.N.J., COLE., D.I., WICKENS., H. D.E V., CHISTIE, A.D.M., ROBERTS, D.L AND BRANDL, G., 2006. Sedimentary of the Karoo Supergroup. *In:* M.R., Johnson, C.R., Anhaeusser and R.J.Thomas (eds). The geology of South Africa

JUPP, P.G., 2005. Mosquitoes as vectors of human disease in South Africa. *South African Family Practice*, 47, 68-72.

KAISER, E.A. AND HEINEMEYER, O., 1993. Seasonal variations of soil microbial biomass carbon within the plough layer. *Soil Biology and Biochemistry*, 25, 1649-1655.

KIMURA, M., JIA, Z.J., NAKAYAMA, N. AND ASAKAWA, S., 2008. Ecology of viruses in soils: past, present and future perspectives. *Soil Science and Plant Nutrition*, 54, 1-32.

KOTZE, D., MARNEWECK, G., BATCHELOR, A., LINDLEY, D. AND COLLINS, N., 2009. WET-EcoServices: a technique for rapidly assessing ecosystem services supplied by wetlands. WRC Report No TT 339/08, Water Research Commission, Pretoria.

KOTZE, D.C. AND MARNEWECK, G.C., 1999. Guidelines for delineating the boundaries of a wetland and the zones within a wetland in terms of the South African Water Act. Department of Water Affairs, Pretoria.

KOTZE, D.C., BREEN, C.M. AND KLUG, J.R., 1994. Wetland-use: A wetland management decision support system for the KwaZulu/Natal Midlands*:* Report to the Water Research Commission, Pretoria.

KOTZE, D.C., KLUG, J.R., HUGHES, J.C. AND BREEN, C.M., 1996. Improved criteria for classifying hydric soils in South Africa. *South African Journal of Plant and Soil*, 13, 67-73.

LABEAUD, A.D., KAZURA, J.W. AND KING, C.H., 2010. Advances in Rift Valley fever research: insights for disease prevention. *Current opinion in infectious diseases*, *23*, 403.

LADD, J.N., BUTLER, J.H.A. AND AMATO, M., 1986. Nitrogen fixation by legumes and their role as sources of nitrogen for soil and crop. *Biological Agriculture and Horticulture*, 3, 269-286.

LE ROUX, P.A.L., HENSLEY, M. AND VAN HUYSSTEEN, C.W. 2010. Advances in pedology in South Africa. *South African Journal of Plant and Soil*, 27, 1-8.

LEVEL LOGGER USER SERIES MANUAL GUIDE, 2018. https://www.fondriest.com/pdf/solinst_gold_manual.pdf. (Accessed 05/09/2019)

LEWIS, W.M., J.R.B., BEDFORD, F., BOSSELMAN III.M BRINSON, P., GARRETT, C., HUNT, C., JOHNSTON, D., KANE, A.M MACRANDER, J. MCCULLEY, M.J MITSCH, W., PATRICK, J.R., R., POST, D., SIEGEL, R.W., SKAGGS, M., STRAND, AND J.B ZEDLER., 1995. Wetlands: characteristics and boundaries. National Academies Press, Washington.

LINTHICUM, K.J., ANYAMBA, A., BRITCH, S.C., CHRETIEN, J.P., BENNETT, K.E., MAYER, R.T., SCHMIDTMANN, E.T., WILSON, W.C., FREIER, J.E., JAMES, A.M. AND MILLER, R.S., 2007. A Rift Valley fever risk surveillance system for Africa using remotely sensed data: potential for use on other continents, *Veterinaria Italiana,* 43, 663-674.

LINTHICUM, K.J., ANYAMBA, A., TUCKER, C.J., KELLEY, P.W., MYERS, M.F. AND PETERS, C.J., 1999. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science*, 285, 397-400.

LINTHICUM, K.J., BAILEY, C.L., DAVIES, F.G. AND TUCKER, C.J., 1987. Detection of Rift Valley fever viral activity in Kenya by satellite remote sensing imagery. *Science*, 235, 1656-1659.

LINTHICUM, K.J., BRITCH, S.C. AND ANYAMBA. A., 2016. Rift Valley fever: An emerging mosquito-borne diseases. *Annual. Review of. Entomology*, 61, 395-415.

LINTHICUM, K.J., DAVIES, F.G., BAILEY, C.L. AND KAIRO, A., 1984. Mosquito species encountered in a flooded grassland *dambo* in Kenya. *Mosquito News,* 44, 228-232.

LOGAN, T.M., LINTHICUM, K.J., THANDE, P.C., WAGATEH, J.N., NELSON, G.O. AND ROBERTS, C.R., 1991. Egg hatching of *Aedes* mosquitoes during successive floodings in a Rift Valley fever endemic area in Kenya. *Journal of the American Mosquito Control Association*, 7, 109-112.

LOTTER, M.C., RUTHERFORD, M.C., BREDENKAMP, G.J. AND POWRIE, L.W., 2006. Grassland biome. Biomes and bioregions of Southern Africa*. In*: L. Mucina, and M.C. Rutherford. (eds), The vegetation of South Africa, Lesotho and Swaziland.

MAES, P., ALKHOVSKY, S.V., BÀO, Y., BEER, M., BIRKHEAD, M., BRIESE, T., BUCHMEIER, M.J., CALISHER, C.H., CHARREL, R.N., CHOI, I.R. AND CLEGG, C.S., 2018. Taxonomy of the family *Arenaviridae* and the order *Bunyavirales*: update 2018. *Archives of virology*, 163, 2295-2310.

MARTIN, V., CHEVALIER, V., CECCATO, P., ANYAMBA, A., DE SIMONE, L., LUBROTH, J., DE LA ROCQUE, S. AND DOMENECH, J., 2008. The impact of climate change on the epidemiology and control of Rift Valley fever. *Revue Scientifique et Technique-Office international des épizooties*, *27*, 413-426.

MASON, S.J., 2001. El Niño, climate change, and Southern African climate. *Environmetrics: The official journal of the International Environmetrics Society*, 12, 327-345.

MCBRIDE, M.B., 1994. Environmental chemistry of soils. Oxford University. Press, New York.

MCCARTNEY, M., REBELO L.M., SELLAMUTTU, S.S. AND DE SILVA, S., 2010. Wetland, agriculture and poverty reduction. Colombo, Sri Lanka: International Water Management Institute. (IWMI Research Report 137). *Colombo: IWMI*.

MCINTOSH, B.M., RUSSELL, D., DOS SANTOS, I. AND GEAR, J.H., 1980. Rift Valley fever in humans in South Africa. *South African medical journal, 58*, 803-806.

MCPHADEN, M.J., ZEBIAK, S.E. AND GLANTZ, M.H., 2006. ENSO as an integrating concept in earth science. *Science*, 314, 1740-1745.

MIRSKI, T., BARTOSZEZE, M. AND BIELAWSKA-DROZD, A., 2011. Impact of climate change on infectious diseases. *Polish Journal Environmental Studies*, 21, 525-523.

MITCH, W.J. AND GOSSELINK, J.G, 2000. Wetlands. (eds). Wiley, New York.

MLINGANO, A.R.I., 2006. Soils of Tanzania and their potential for agriculture development. Draft report. Mlingano Agricultural Research Institute, Ministry of Agriculture. Food Security and Cooperatives, Tanga.

MUCINA, L. AND RUTHERFORD, M.C., 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19., (South African National Biodiversity Institute: Pretoria, South Africa). Memoirs of the Botanical Survey of South Africa.

MUNYUA, P.M., MURITHI, R.M., ITHONDEKA, P., HIGHTOWER, A., THUMBI, S.M., ANYANGU, S.A., KIPLIMO, J., BETT, B., VRIELING, A., BREIMAN, R.F. AND NJENGA, M.K., 2016. Predictive factors and risk mapping for Rift Valley fever epidemics in Kenya. *Public Library of Science one*, *11*.

NATIONAL RESEARCH COUNCIL, 1995. Wetlands: characteristics and boundaries. Committee on characterization of wetlands, 328.

NATIONAL WATER ACT 36 OF 1998. Government Gazette, Government Printing Works, Cape Town.

NETTERBERG, F., 1978. Dating and correlation of calcretes and other pedocretes. *Transactions of the Geological Society of South Africa*, 81, 379-391.

NEWTON, J.D., WYATT, F.A., IGNATIEFF, V. AND WARD, A.S., 1939. Nitrification under and after alfalfa, brome, timothy, and western rye grass: II. Soil microbiological activity. *Canadian Journal of Research*, 17, 256-293.

NJENGA, M.K. AND BETT, B., 2019. Rift Valley fever virus-how and where virus is maintained during inter-epidemic periods. *Current Clinical Microbiology Reports*, 6, 18-24.

NON-AFFILIATED SOIL ANALYSIS WORK COMMITTEE, 1990. Handbook of standard soil testing methods for advisory purposes. *Soil Science Society of South Africa, Pretoria*, *160*.

OCHIENG, A.O., NANYINGI, M., KIPRUTO, E., ONDIBA, I.M., AMIMO, F.A., OLUDHE, C., OLAGO, D.O., NYAMONGO, I.K. AND ESTAMBALE, B.B., 2016. Ecological niche modelling of Rift Valley fever virus vectors in Baringo, Kenya. *Infection ecology and epidemiology*, 6, 32322.

OFULLA, A.V.O., KARANJA, D., OMONDI, R., OKURUT, T., MATANO, A., JEMBE, T., ABILA, R., BOERA, P. AND GICHUKI, J., 2010. Relative abundance of mosquitoes and snails associated with water hyacinth and hippo grass in the Nyanza gulf of Lake Victoria. *Lakes and Reservoirs: Research and Management*, *15*, 255-271.

OIE, 2010. Follow-Up Report 17 Free State Bultfontein, Paris: OIE; No. 17, OIE Ref: 9982.

OLLIS, D., SNADDON, K., JOB, N. AND MBONA, N., 2013. *Classification system for wetlands and other aquatic ecosystems in South Africa*. South African National Biodiversity Institute.

ONG, G.H., YAP, C.K., MAHMOOD, M., TAN, S.G. AND HAMZAH, S., 2013. Barium levels in soils and Centella asiatica. *Tropical life sciences research*, 24, 55.

OYEDEJI, T., 2015. Application of Discriminant Function Analysis in Agricultural Extension Research.

PANIKOV, N.S., 1999. Understanding and prediction of soil microbial community dynamics under global change. *Applied Soil Ecology*, 11, 161-176.

PARVIN, N. AND DEGRÉ, A., 2016. Soil-specific calibration of capacitance sensors considering clay content and bulk density. *Soil Research*, 54, 111-119.

PEPIN, M., BOULOY, M., BIRD, B.H., KEMP, A. AND PAWESKA J., 2010. Rift Valley fever virus (*Bunyaviridae:Phelobovirus*): an update on pathogenesis, molecular epidemiology vectors, diagnostic and prevention. *Veterinary Research*, 41, 2-40.

PIERZYNSKI, G.M., 2000. Methods of phosphorus analysis for soils, sediments, residuals, and waters.

PONNUSAMY, L., BOOROOCZKY, K., WESSON, D.M., SCHAL, C. AND APPERSON, C.S., 2011. Bacteria stimulate hatching of yellow fever mosquito eggs. *Public Library of Science one*, 6.

POTTS, P.J., 2004. X-Ray Fluoresence Analysis. *In:* K.A. Smith and M.S. Cresser (eds). Soil and Environmental Analysis: modern instrumental techniques. Marcel Dekker. New York.

POULSEN, J. AND FRENCH, A., 2008. Discriminant function analysis. San Francisco State University: San Francisco, CA. http://userwww.sfsu.edu/efc/classes/biol710/discrim/discrim.pdf (Accessed 07/02/2018)

RAMAYAH, T., AHMAD, N.H., HALIM, H.A. AND MAY-CHIUN, S.R.M.Z., 2010. Discriminant analysis: An illustrated example. *African Journal of Business Management*, 4, 1654.

REDDY, K.R., D'ANGELO, E.M. AND HARRIS, W.G., 2000. Biogeochemistry of wetlands. *Handbook of Soil Science*, 89-119.

RIFT VALLEY FEVER OUTBREAKS FORECASTING MODELS, 2009. https://www.who.int/csr/resources/publications/WHO_HSE_GAR_BDP_2009_2/en/. (Accessed 07/02/2018)

ROLIN, A.I., BERRANG-FORD, L. AND KULKARNI, M.A., 2013. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. *Emerging microbes and infections,* 2, 1-8.

ROZEBOOM, L.E., 1934. The effect of bacteria on the hatching of mosquito eggs. *American Journal of Hygiene*, 20, 2.

RUTHERFORD, M.C, MUCINA, L. AND POWRIE, L.W., 2006. Biomes and bioregions of Southern Africa. *In:*, L. Mucina and M.C. Rutherford (eds). The vegetation of South Africa, Lesotho and Swaziland.

SAS INSTITUTE INC (2013). SAS/STAT 13.1 User's Guide. Cary NC: SAS Institute Inc.

SCHNÜRER, J. AND ROSSWALL, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and environmental microbiology*, 43, 1256-1261.

SCHULZE, D.G., 2002. An introduction to soil mineralogy. *In:* J.B. Dixon and D.G. Schulze (eds). Soil mineralogy with environmental applications. Soil Science Society of America. Madison.

SCHUYT, K.D., 2005. Economic consequences of wetland degradation for local populations in Africa. *Ecological Economics*, 53,177–190.

SHAWKY. S., 2000. Rift Valley fever. *Saudi Medical Journal.* 21, 1109-1115.

SISSOKO, D., GIRY C., GABRIE, P., TARANTOLA A., PETTINELLI, F., COLLET, L., D'ORTENZIO, E., RENAULT, P. AND PIERRE V., 2009. Rift Valley fever, Mayotte, 2007-2008. *Emerging infectious diseases*, 15, 568-570.

SMITH, K. AND VAN HUYSSTEEN, C.W., 2011. The effect of degree and duration of water saturation on selected redox indicators: pe, Fe^{2+} and Mn^{2+}. *South African Journal of Plant and Soil*, 28, 119-126.

SOIL CLASSIFICATION WORKING GROUP. 1991. Soil classification- a taxonomic system for South Africa. Memoirs on the Agricultural Natural Resources of South Africa No. 15. Department of Agricultural Development, Pretoria.

SPARKS, D.L., 2003. Environmental soil chemistry. Elsevier science, USA.

SPOSITO, G., 1989. The chemistry of soils. Oxford University Press, New York.

STARR, J.L. AND PALTINEANU, I.C., 2002. Methods for measurement of soil water content: capacitance devices. *Methods of soil analysis: part*, *4*.

STORDY, R.J., 1913. Annual Report Department of Agriculture, British East Africa: 1912–1913.

SWANEPOEL, R. AND COETZER, J.A.W., 2004. Rift Valley fever. *In:* J.A.W. Coetzer and R.C. Tustin (eds.), *Infectious diseases of livestock with special reference to Southern Africa*, 1037–1070, Oxford University Press, Cape Town.

SZOGI, A.A, HUNT, P.G, SADLER, E.J. AND EVANS, D.E., 2004. Characterization of oxidation-reduction processes in constructed wetlands for swine wastewater treatment. *American Society of Agricultural Engineer*, 20, 189-200.

TAYLOR, D., HAGENLOCHER, M., JONES, A.E., KIENBERGER, S., LEEDALE, J. AND MORSE, A.P., 2016. Environmental change and Rift Valley fever in Eastern Africa: projecting beyond healthy futures. *Geospatial health*, 11, 115-128

THE RAMSAR CONVENTION MANUAL, 2008. A guide to the Convention on wetlands 4th edition.

THOMPSON, P.N. AND PIENAAR, N.J., 2013. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. *Onderstepoort Journal of Veterinary Research*, 80, 1-13.

TINER, R.W., 1993. Using plants as indicators of wetlands. *Proceedings of the Academy of Natural Science of Philidelphia,* 144, 240-253.

TINER, R.W., 1999. Wetland indicators. A guide to wetland identification, delineation, classification, and mapping. Lewis Publishers/CRC Press, Boca Raton, Florida.

TURELL, M.J., LINTHICUM, K.J., PATRICAN, L.A., DAVIES, F.G., KAIRO, A. AND BAILEY, C.L., 2008. Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *Journal of Medical Entomology*, 45, 102-108.

TURNER D 1991. Minimum data set for describing soil profiles. Institute for Soil, Climate and Water, Pretoria, South Africa.

U.S. ARMY CORPS OF ENGINEERS, 1987. Corps of Engineers Wetland Delineation Manual.

U.S. ARMY CORPS OF ENGINEERS, 2012. Regional Supplement to the Corps of Engineers Wetland Delineation Manual: Northcentral and Northeast region.

VAN GESTEL, M., LADD, J.N. AND AMATO, M., 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. *Soil Biology and Biochemistry*, 24, 103-111.

VAN HUYSSTEEN, C.W., HENSLEY, M., LE ROUX, P.A.L., ZERE, T.B. AND DU PREEZ, C.C., 2005. The relationship between soil water regime and soil profile morphology in the Weatherley catchment, an afforestation area in the North-Eastern Eastern Cape. *Water Research Commission (WRC) Report*, (K5/1317) WRC Pretoria.

VAN HUYSSTEEN, C.W., ZERE, T.B. AND HENSLEY, M., 2010. Soil-water relationships in the Weatherley catchment, South Africa. *Water SA*, 36, 521-530.

VAN VUREN, P.J., KGALADI, J., PATHAROO, V., OHAEBOSIM, P., MSIMANG, V., NYOKONG, B. AND PAWESKA, J.T., 2018. Human cases of Rift Valley fever in South Africa, 2018. *Vector-Borne and Zoonotic Diseases*, *18*, 713-715.

VEPRASKAS, M.J., RICHARDSON, J.L. AND TANDARICH, J.P., 2006. Dynamics of redoximorphic feauture formation under controlled ponding in a created riverine wetland. *The Society wetlands scientist*, 26, 486-496.

VEPRASKAS, M.J., RICHARDSON, J.L., TANDARICH, J.P. AND Teets, S.J., 1999. Dynamics of hydric soil formation across the edge of a created deep marsh. *The Society wetlands scientist*, 19, 78-89.

VEPRASKAS, M.J. 2001. Morphological features of seasonally reduced soils. *In:* J. L. Richardson and M. J. Vepraskas (eds). Wetland soils: genesis, hydrology, landscapes, and classification. Washington.

VEPRASKAS, M.J. AND FAULKNER, S.P., 2001. Redox Chemistry of Hydric Soils. *In:* J.L. Richardson and M.J. Vepraskas (eds). Wetland soils: genesis, hydrology, landscapes, and classification. Washington.

VEPRASKAS, M.J., RICHARDSO. J.L. AND TANDARICH, J.P., 1995. Development of Redoximorphic Features in Constructed Wetland Soils. *Chicago, IL: Wetlands Research Inc*

VEPRASKAS, M.J., S.J. TEETS, J.L. RICHARDSON, AND TANDARICH, J.P., 1995. Development of redoximorphic features in constructed wetland soils. Technical paper . Wetlands Research, Inc.

VERSTER, M. 2016. Wetland soil properties conducive to the aestivation of Rift-Valley fever infected *Aedes* mosquitos in Central South Africa. M.Sc. Thesis, University of the Free State, Bloemfontein, South Africa.

VERSTER, A.M., LIANG, J.E., ROSTAL, M.K., KEMP, A., BRAND, R.F., ANYAMBA, A., CORDEL, C., SCHALL, R., ZWIEGERS, H., PAWESKA, J.T. AND KARESH, W.B., 2020. Selected wetland soil properties correlate to Rift Valley fever livestock mortalities reported in 2009-10 in central South Africa. *PlosOne*, 15, 5. e0232481.

WATER MANUAL, 2001. https://www.irrometer.com/pdf/instruction-manuals/sensors/701 Meter Manual-WEB.pdf (Accessed 06/7/2019)

WILLIAMS, R., MALHERBE, J., WEEPENER, H., MAJIWA, P. AND SWANEPOEL, R., 2016. Anomalous high rainfall and soil saturation as combined risk indicator of Rift Valley fever outbreaks, South Africa, 2008-2011. *Emerging Infectious Diseases*, 22, 2052-2062.

WILLIS, J.P. AND DUNCAN, A.R., 2008. Understanding XRF Spectrometry, Volume 2: Quantitative analysis and special sample preparation and presentation methods. *Almelo: Malvern Panalytical,* 13, 11.

WIRELESS SOIL MOISTURE/ TEMPERATURE STATION INSTALLATION MANUAL, 2001. https://www.davisinstruments.com/product_documents/weather/manuals/soil_moisture_manual.pdf. (Accessed 08/9/2019)

WHO (WORLD HEALTH ORGANISATION), 2017. Vector borne diseases. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases. (Accessed 06/9/2019)

OIE (World Organization for Animal Health), 2018. Rift Valley fever, South Africa. https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=26639. (Accessed 06/3/2019)

WU, X., LU, Y., ZHOU, S., CHEN, L. AND XU, B., 2016. Impact of climate change on human infectious diseases: Empirical evidence and human adaptation. *Environment international*, 86, 14-23.

WU, X., TIAN, H., ZHOU, S., CHEN, L. AND XU, B., 2014. Impact of global change on transmission of human infectious diseases. *Science China Earth Sciences*, 57,189-203.

WUANA, R.A. AND OKIEIMEN, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Network Ecology.

Appendix A: Soil profile description of the new study sites

Profile No: 321hoppplr
Latitude & Longitude: -29.79107 / 24.17594
Surface stoniness: none
Terrain unit: closed depression
Slope shape: concave
Aspect: level
Microrelief: none
Parent material: single; alluvium
Underlying material: Igneous rocks: Basic (basalt, dolerite and diabase)
Alteration of underlying material: calcified
Geological Group:

Soil form: Addo
Soil family: 2112
Surface rockiness: none
Occurrence of flooding: occasional
Wind erosion: none
Water erosion: none
Vegetation / Land use: marsh
Water table: not reached
Described by: Z Gqalaqha & CW van Huyssteen
Date described: 03/2017
Weathering of underlying material: moderate

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR7/2 (100%); **moist colour:** 7.5YR.3/1 (100%); clay loam; no mottles; structure is moderate angular blocky; soft, firm, very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 1 second(s); many roots; difus smooth transition;	Topsoil
A2	50 - 100	**Moisture status:** moist; **dry colour:** 7.5YR8/1 (100%); **moist colour:** 7.5YR7/1 (100%); clay loam; few faint mottles; moderate angular blocky; very hard, firm, very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 1 second(s); few roots; difus smooth transition;	Orthic A
B1	100 – 500	**Moisture status:** moist; **dry colour:** 7.5YR8/1 (100%); **moist colour:** 7.5YR6/1 (100%); clay; few faint mottles; moderate angular blocky; very hard, firm, very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 1 second(s); few roots; difus smooth transition;	Neocarbonate B
B2	500 – 1200	**Moisture status:** moist; **dry colour:** 7.5YR8/1 (100%); **moist colour:** 7.5YR6/1 (100%);clay; few faint mottles; moderate angular blocky; very hard, firm, very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 1 second(s); few roots; nrech smooth transition;	Soft carbonate

Profile No: 131222phibthl
Latitude & Longitude: -30.11202 / 25.12394
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: concave
Aspect: level
Microrelief: none
Parent material: alluvium
Underlying material: Igneous rocks: Basic (basalt, dolerite and diabase)
Alteration of underlying material: ferruginised
Geological Group:

Soil form: Addo
Soil family: 1112
Surface rockiness: none
Occurrence of flooding: none
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: not reached
Described by: Z Gqalaqha & CW van Huyssteen
Date described: 03/2017
Weathering of underlying material: strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR5/2 (100%); **moist colour:** 7.5YR.3/2 (100%); silty clay; no mottles; structure is weak subangular blocky; soft, slightly firm,non-sticky, non-plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 0 second(s); few roots; clear smooth transition;	Topsoil
A2	50 - 300	**Moisture status:** moist; **dry colour:** 7.5YR5/2 (100%); **moist colour:** 7.5YR3/2 (100%); silty clay; no mottles; weak subangular blocky; soft, slightly firm,non-sticky, non-plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 0 second(s); few roots; clear smooth transition;	Orthic A
B1	300 – 600	**Moisture status:** moist; **dry colour:** 7.5YR5/1 (100%); **moist colour:** 7.5YR5/2 clay; no mottles; weak subangular blocky; soft, slightly firm,non-sticky, non-plastic; few slickensides; no cracks; no cutans; no coarse fragments; water absorption 2 second(s); common roots; clear smooth transition;	Neocarbonate B
B2	600 – 1200	**Moisture status:** moist; **dry colour:** 7.5YR7\8/1(100%); **moist colour:** 7.5YR6/2 (100%); clay; few prominent olive mottles; apedal massive; slightly hard, firm, non-sticky, non- plastic; no slickensides; no cracks; few cutans; no coarse fragments; water absorption 3 second(s); common roots; nrech smooth transition;	Soft carbonate

Profile No: 259edemdws
Latitude & Longitude: -29.78264 / 25.94093
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: concave
Aspect: level
Microrelief: none
Parent material: binary; alluvium
Underlying material: Igneous and sedimentary rock: (dolerite and shale)
Alteration of underlying material: ferruginised
Geological Group:

Soil form: Longlands
Soil family: 1000
Surface rockiness: none
Occurrence of flooding: frequent
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: not reached
Described by: Z Gqalaqha & CW van Huyssteen
Date described: 03/2017
Weathering of underlying material: strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 300	**Moisture status:** moist; **dry colour:** 7.5YR4/1 (100%); **moist colour:** 7.5YR.3/1 (100%); silty clay; no mottles structure is moderate subangular blocky; slightly hard, slightly firm, slightly sticky, slightly plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 4 second(s); no roots; clear smooth transition;	Topsoil
A2	300 - 500	**Moisture status:** moist; **dry colour:** 7.5YR5/1 (100%); **moist colour:** 7.5YR2/1 (100%); clay; few faint red mottles; moderate subangular blocky; slightly hard, slightly firm, sticky, slightly plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 4 second(s); no roots; clear smooth transition;	Orthic A
B1	500 – 800	**Moisture status:** moist; **dry colour:** 7.5YR5/1 (100%); **moist colour:** 7.5YR2/1 (100%); silty clay; common distinct grey and yellow mottles; strong subangular blocky; slightly hard, slightly firm, sticky, plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 1 second(s); no roots; clear smooth transition;	E
B2	800 – 1200	**Moisture status:** moist; **dry colour:** 7.5YR7/1 (100%); **moist colour:** 7.5YR5/3 (100%); clay; many prominent grey, yellow and olive mottles are common; moderate granular; soft, friable, slightly sticky, plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; clear smooth transition;	Soft plinthic B

Profile No: 211bosxcls
Latitude & Longitude: -28.65843 / 25.19342
Surface stoniness: none
Terrain unit: footslope
Slope shape: straight
Aspect: level
Microrelief: none
Parent material: unkown
Underlying material: Igneous rocks: Basic (basalt, dolerite and diabase)
Alteration of underlying material: calcified
Geological Group:

Soil form: Augrabies
Soil family: 1110
Surface rockiness: none
Occurrence of flooding: none
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: not reached
Described by: Z Gqalaqha & T Setsepane
Date described: 04/2017
Weathering of underlying material: strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR3/3 (100%); **moist colour:** 7.5YR.4/3 (100%); sandy clay loam; common distinct black,grey and red mottles; structure is weak subangular blocky; soft, friable,non-sticky, non plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 3 second(s); few roots; gradual smooth transition;	Topsoil
A2	50 - 200	**Moisture status:** moist; **dry colour:** 7.5YR3/3 (100%); **moist colour:** 7.5YR4/3 (100%); sand clay loam; common distinct black,grey and red mottles; weak subangular blocky; soft, friable,non-sticky, non plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 3 second(s); few roots; gradual smooth transition;	Orthic A
E1	200 – 500	**Moisture status:** moist; **dry colour:** 7.5YR5/3 (100%); **moist colour:** 7.5YR4/4 (100%); sandy clay loam; few faint black, grey and red mottles; apedal single grain; loose, loose, non-sticky, non-plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 0 second(s); no roots; gradual smooth transition;	Neocarbonate B
E2	500 – 700	**Moisture status:** moist; **dry colour:** 7.5YR6/6(100%); **moist colour:** 7.5YR5/4 (100%); sandy clay loam; no mottles; apedal single grain; loose, loose, non- sticky, non-plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 0 second(s); no roots; gradual smooth transition;	Unspecified

Profile No: 284petkrfs
Latitude & Longitude: -29.23234 / 25.51056
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: concave
Aspect: level
Microrelief: none
Parent material: alluvium
Underlying material: Igneous rocks: Basic (basalt, dolerite and diabase)
Alteration of underlying material: ferruginised
Geological Group:

Soil form: Augrabies
Soil family: 1210
Surface rockiness: none
Occurrence of flooding: occasional
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: high
Described by: Z Gqalaqha & Setsepane
Date described: 04/2017
Weathering of underlying material: strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR6/1 (100%); **moist colour:** 7.5YR.5/1 (100%); sandy clay loam; no mottles; structure is moderate subangular blocky; soft, slightly firm,sticky, plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; gradual smooth transition;	Topsoil
A2	50 - 300	**Moisture status:** moist; **dry colour:** 7.5YR6/1 (100%); **moist colour:** 7.5YR5/1 (100%);clay; no mottles; moderate subangular blocky; soft, slightly firm,sticky, plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; gradua smooth transition;	Orthic A
B1	300 – 600	**Moisture status:** moist; **dry colour:** 7.5YR5/3 (100%); **moist colour:** 7.5YR5/1 (100%);clay; few faint black and red mottles; moderate angular blocky; soft, slightly firm, very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 5 second(s); no roots; gradual smooth transition;	Neocarbonate B
B2	600 – 1200	**Moisture status:** moist; **dry colour:** 7.5YR6/4(100%); **moist colour:** 7.5YR3/6 (100%);clay; few faint black and red mottles; moderate subangular blocky; soft,friable, slightly sticky, slightly plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 5 second(s); no roots; gradual smooth transition;	Unspecified

Profile No: 131smibssv#1
Latitude & Longitude: -30.12898 / 26.41504
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: straight
Aspect: level
Microrelief: none
Parent material: binary; alluvium and solid rock
Underlying material: sedimentary rocks (shale and tillite)
Alteration of underlying material: ferruginised
Geological Group:

Soil family: 1000
Soil form: Longlands
Surface rockiness: none
Occurrence of flooding: occasional
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: not reached
Described by: Z Gqalaqha & CW van Huyssteen
Date described: 03/2017
Weathering of underlying material: advanced strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR3/2 (100%); **moist colour:** 7.5YR.6/2 (100%); clay; no mottles; structure is weak granular; loose, loose,non-sticky, non plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 6 second(s); no roots; clear smooth transition;	Topsoil
A2	50 - 200	**Moisture status:** moist; **dry colour:** 7.5YR3/2 (100%); **moist colour:** 7.5YR6/1 (100%); clay; few faint red mottles ; moderate angular blocky; soft, friable, non-sticky, non- plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 6 second(s); no roots; clear smooth transition;	Orthic A
B1	200 – 500	**Moisture status:** moist; **dry colour:** 7.5YR3/1 (100%); **moist colour:** 7.5YR5/1 (100%); clay; common prominent red, grey and yellow mottles; strong subangular blocky; hard, firm, slightly sticky, slightly plastic; no slickensides; fine cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; clear smooth transition;	E
B2	500 – 700	**Moisture status:** moist; **dry colour:** 7.5YR5/4 (100%); **moist colour:** 7.5YR7/3 (100%); clay; many prominent red, black, brown,grey and yellow mottles; strong subangular blocky; hard, firm, slightly sticky, slightly plastic; few slickensides; fine cracks; no cutans; no coarse fragments; water absorption 1 second(s); no roots; clear smooth transition;	Soft plinthic B

Profile No: 131smibssv#2
Latitude & Longitude: -30.10898 / 26.14150
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: straight
Aspect: level
Microrelief: none
Parent material: binary; alluvium
Underlying material: sedimentary rocks (shale and tillite)
Alteration of underlying material: ferruginised
Geological Group:

Soil form: Kastpruit
Soil family: 1000
Surface rockiness: none
Occurrence of flooding: frequent
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: high
Described by: Z Gqalaqha & CW van Huyssteen
Date described: 03/2017
Weathering of underlying material: strong

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 150	**Moisture status:** moist; **dry colour:** 7.5YR7/1 (100%); **moist colour:** 7.5YR.2/5 (100%); clay; no mottles; structure is moderare subangular blocky; slightly hard, slightly firm,very sticky, very plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 4 second(s); no roots; clear smooth transition;	Topsoil
G1	150 - 600	**Moisture status:** moist; **dry colour:** 7.5YR6/2 (100%); **moist colour:** 7.5YR5/1 (100%); clay; common distinct olive, grey and yellowmottles ; moderate angular blocky; soft, friable, non-sticky, non- plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; clear smooth transition;	G
G2	600 – 1200	**Moisture status:** moist; **dry colour:** 7.5YR7/2 (100%); **moist colour:** 7.5YR6/1 (100%); clay; many prominent red, black,grey, yellow and olive mottles; strong subangular blocky; hard, firm, slightly sticky, slightly plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 1 second(s); no roots; clear smooth transition;	G

Profile No: 140troklkn
Latitude & Longitude: -29.96489 / 26.14865
Surface stoniness: none
Terrain unit: valley bottom
Slope shape: concave
Aspect: level
Microrelief: none
Parent material: binary; alluvium
Underlying material: Igneous rocks: Intermidiate (diorite, granodiorite)
Alteration of underlying material: ferruginised
Geological Group:

Soil form: Augrabies
Soil family: 1100
Surface rockiness: none
Occurrence of flooding: occasional
Wind erosion: none
Water erosion: none
Vegetation / Land use: Grassveld open
Water table: not reached
Described by: Z Gqalaqha & T Setsepane
Date described: 04/2017
Weathering of of underlying material: unkown

Horizon	Depth (mm)	Description	Diagnostic horizons
A1	0 – 50	**Moisture status:** moist; **dry colour:** 7.5YR5/1 (100%); **moist colour:** 7.5YR.3/1 (100%); clay; common distictinct black, olive and red mottles; structure is moderate angular blocky; slightly hard, slightly firm, slightly sticky, slightly plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 3 second(s); few roots; gradual smooth transition;	Topsoil
A2	50 - 300	**Moisture status:** moist; **dry colour:** 7.5YR5/1 (100%); **moist colour:** 7.5YR3/1 (100%); clay; common distictinct black, olive and red mottles; moderate angular blocky; slightly hard, slightly firm, slightly sticky, slightly plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 3 second(s); few roots; gradual smooth transition;	Orthic A
B1	300 – 600	**Moisture status:** moist; **dry colour:** 7.5YR5/6 (100%); **moist colour:** 7.5YR3/3 (100%); clay; many prominent black, olive and red mottles; moderate angular blocky; slightly hard, slightly firm, sticky, plastic; few slickensides; no cracks; no cutans; no coarse fragments; water absorption 2 second(s); no roots; gradual smooth transition;	Neocarbonate B
B2	600 – 1000	**Moisture status:** moist; **dry colour:** 7.5YR4/8 (100%); **moist colour:** 7.5YR3/3 (100%); silty clay; many prominent black, olive and red mottles; weak subangular blocky; soft, friable, sticky, slightly plastic; no slickensides; no cracks; no cutans; no coarse fragments; water absorption 5 second(s); no roots; gradual smooth transition;	Unspecified

Appendix B: Soil chemical characterisation of the study sites

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
N		13	8	9	13	7	9	13	8	9	12	8	6
Organic Carbon (%)	Mean	3.55	2.61	3.32	2.54	1.02	2.13	2.32	0.63	2.92	2.62	4.49	3.63
	Std	2.33	1.65	1.75	1.88	5.49	9.46	1.83	0.48	2.24	2.03	2.74	1.75
	Min	5.10	8.90	1.00	4.10	2.77	7.70	0.54	0.04	4.80	6.50	5.18	1.84
	Median	3.12	2.30	3.06	2.00	8.83	1.97	1.82	0.54	2.10	1.97	3.68	3.26
	Max	7.67	6.05	6.39	5.55	1.95	4.22	7.22	1.64	6.71	6.78	8.65	6.91
Total Nitrogen (%)	Mean	2.29	-	2.45	1.19	-	1.24	9.49	-	6.93	7.68	-	5.37
	Std	1.39	-	1.20	6.63	-	3.70	6.86	-	2.77	5.25	-	2.29
	Min	3.00	-	1.14	2.00	-	7.70	2.20	-	3.30	1.80	-	2.40
	Median	2.24	-	1.67	9.90	-	1.13	6.30	-	7.20	5.80	-	5.95
	Max	4.74	-	4.05	2.36	-	1.79	2.60	-	1.09	1.92	-	8.70
Calcium Chloride ($CaCl_2$) Phosphorus (mg kg^{-1})	Mean	0.40	0.60	0.13	0.22	0.31	0.08	0.30	0.83	0.11	0.23	0.56	0.14
	Std	0.27	0.59	0.06	0.16	0.26	0.02	0.24	1.07	0.04	0.17	0.60	0.08
	Min	0.08	0.10	0.07	0.04	0.12	0.06	0.05	0.11	0.06	0.02	0.09	0.05
	Median	0.44	0.52	0.10	0.15	0.25	0.08	0.28	0.40	0.11	0.21	0.44	0.11
	Max	0.86	1.97	0.25	0.49	0.89	0.11	0.86	3.13	0.18	0.49	1.98	0.26
Water extraction Phosphorus (mg kg^{-1})	Mean	2.01	2.48	0.89	1.52	2.01	1.03	0.70	1.64	0.58	0.78	1.86	0.43
	Std	3.07	2.24	0.72	2.16	2.28	1.02	0.44	2.20	0.45	1.12	3.12	0.16
	Min	0.16	0.41	0.16	0.26	0.32	0.16	0.25	0.18	0.06	0.12	0.18	0.23
	Median	0.64	1.90	0.80	0.63	1.25	0.38	0.64	0.90	0.40	0.50	0.88	0.44
	Max	11.51	6.73	2.48	6.73	6.95	2.53	1.99	6.92	1.60	4.25	9.53	0.67

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
N		13	8	9	13	7	9	13	8	9	12	8	6
Olsen Phosphorus (mg kg^{-1})	Mean	26.25	32.48	28.52	12.72	21.46	12.32	8.03	14.33	4.14	7.67	11.85	1.04
	Std	15.05	21.06	23.48	10.14	17.08	6.36	12.69	15.70	4.00	13.44	12.28	1.56
	Min	4.48	2.45	8.79	0.00	1.68	4.34	0.00	0.38	0.00	0.00	0.47	0.00
	Median	22.03	40.67	22.86	13.68	16.70	11.35	5.25	9.07	4.86	2.75	7.42	1.33
	Max	51.10	57.66	86.57	34.12	55.47	20.99	47.75	51.40	10.08	43.21	37.28	2.61
pH_{KCl}	Mean	7.30	6.21	6.97	7.11	6.56	7.26	7.14	6.87	7.68	7.17	7.09	8.03
	Std	1.89	1.20	0.89	1.44	1.29	0.92	1.09	1.17	0.80	0.89	1.10	0.51
	Min	4.15	3.76	4.86	4.65	4.06	5.05	5.16	4.39	6.01	5.50	4.55	7.41
	Median	7.21	6.53	7.23	7.29	6.99	7.41	7.38	7.30	7.65	7.40	7.50	7.96
	Max	10.00	7.31	7.75	9.35	7.63	8.21	8.82	7.91	8.84	8.54	7.92	8.91
pH_{water}	Mean	8.13	7.27	7.85	8.26	7.81	8.23	8.19	8.15	8.58	8.25	8.42	8.77
	Std	1.52	0.91	0.93	1.15	1.22	0.95	0.82	1.15	0.84	0.73	1.10	0.75
	Min	5.44	5.51	5.75	6.37	5.62	6.28	7.11	5.87	7.38	6.96	6.06	8.16
	Median	7.92	7.74	7.99	8.08	8.24	8.08	8.14	8.47	8.22	8.22	8.71	8.39
	Max	10.43	7.99	9.08	10.02	9.30	9.46	9.77	9.48	10.04	9.64	9.66	9.98
CEC ($cmol_c$ kg^{-1})	Mean	19.92	23.11	30.12	16.33	18.78	27.38	16.31	16.51	26.84	21.04	16.84	19.06
	Std	11.96	10.08	8.72	9.32	12.43	9.01	7.96	8.86	11.58	10.64	8.13	18.32
	Min	3.83	4.46	18.00	4.17	1.63	17.39	4.00	1.10	15.30	5.39	1.23	1.04
	Median	16.35	26.03	32.17	13.91	16.13	26.96	15.91	16.73	23.48	20.26	18.12	12.61
	Max	40.00	34.34	41.74	36.52	42.38	40.00	34.78	33.65	47.83	37.39	27.09	52.17
Exchangeable Ca^{2+} ($cmol_c$ kg^{-1})	Mean	24.16	26.91	47.61	26.60	26.98	44.64	27.57	30.34	48.19	27.25	33.80	50.15
	Std	12.54	18.15	16.11	11.16	14.88	12.71	10.94	16.54	10.99	13.08	14.55	10.25
	Min	6.50	12.94	14.21	7.15	10.05	17.51	10.25	8.61	32.63	3.53	7.88	35.63
	Median	26.40	18.43	48.63	29.90	25.03	49.63	29.38	29.50	52.50	29.70	38.05	53.25
	Max	38.50	55.70	70.50	40.40	51.89	61.00	49.75	52.50	61.50	47.50	50.85	61.38

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
N		13	8	9	13	7	9	13	8	9	12	8	6
Exchangeable K^+ ($cmol_c\ kg^{-1}$)	Mean	1.87	1.79	3.36	1.48	1.41	3.25	1.53	1.10	3.14	1.28	0.83	3.18
	Std	0.86	0.38	1.91	0.79	0.40	1.25	0.86	0.36	1.33	0.67	0.25	2.16
	Min	0.98	1.15	0.13	0.46	0.77	1.92	0.23	0.70	1.29	0.40	0.60	1.27
	Median	1.52	1.87	2.88	1.17	1.49	2.94	1.52	1.08	3.25	1.30	0.76	2.46
	Max	3.84	2.44	6.20	2.99	1.83	5.95	3.70	1.73	5.69	2.75	1.33	7.29
Exchangeable Mg^{2+} ($cmol_c\ kg^{-1}$)	Mean	7.86	10.06	11.74	7.33	9.46	9.98	8.11	12.05	11.06	10.38	12.74	11.60
	Std	6.66	4.69	5.19	4.45	3.61	4.01	5.11	5.94	4.95	7.23	7.18	6.63
	Min	1.18	5.40	6.88	2.50	5.25	5.00	2.83	5.27	5.42	2.83	4.75	5.21
	Median	5.00	8.86	11.46	5.33	9.14	8.96	5.50	10.29	11.88	7.17	9.29	10.83
	Max	21.04	18.69	23.75	13.33	15.31	15.21	19.67	21.50	21.67	23.96	24.35	23.96
Exchangeable Na^+ ($cmol_c\ kg^{-1}$)	Mean	2.43	0.92	5.35	1.95	0.71	10.57	1.48	1.62	9.49	1.65	2.20	11.59
	Std	2.92	1.17	9.20	1.92	0.50	15.95	1.66	1.76	13.04	1.48	2.29	18.56
	Min	0.18	0.21	0.12	0.13	0.21	0.09	0.14	0.19	0.08	0.15	0.22	0.07
	Median	0.82	0.30	0.54	1.99	0.48	2.52	0.77	1.30	3.05	1.29	1.66	1.78
	Max	8.26	3.60	26.09	5.74	1.48	43.04	5.22	5.78	36.30	4.35	6.83	46.20
Soluble Ca^{2+} ($cmol_c\ kg^{-1}$)	Mean	0.17	0.23	0.60	0.19	0.18	0.58	0.15	0.19	0.40	0.10	0.19	0.35
	Std	0.12	0.09	0.38	0.27	0.06	0.44	0.21	0.08	0.43	0.07	0.08	0.18
	Min	0.01	0.13	0.20	0.03	0.10	0.20	0.03	0.09	0.12	0.02	0.08	0.10
	Median	0.15	0.22	0.55	0.14	0.20	0.46	0.09	0.19	0.25	0.09	0.18	0.33
	Max	0.35	0.43	1.49	1.06	0.25	1.57	0.83	0.34	1.49	0.27	0.30	0.60
Soluble K^+ ($cmol_c\ kg^{-1}$)	Mean	0.02	0.06	0.01	0.00	0.04	0.01	0.00	0.06	0.02	0.00	0.10	0.03
	Std	0.03	0.04	0.00	0.00	0.02	0.01	0.00	0.03	0.04	0.01	0.11	0.05
	Min	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00
	Median	0.00	0.05	0.01	0.00	0.03	0.01	0.00	0.05	0.00	0.00	0.05	0.00
	Max	0.10	0.15	0.01	0.02	0.08	0.03	0.01	0.12	0.12	0.03	0.32	0.14

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
N		13	8	9	13	7	9	13	8	9	12	8	6
Soluble Mg^{2+} ($cmol_c\ kg^{-1}$)	Mean	0.33	0.09	0.03	0.10	0.05	0.05	0.04	0.07	0.39	0.02	0.07	0.68
	Std	0.87	0.09	0.02	0.25	0.02	0.06	0.06	0.07	1.12	0.02	0.05	1.61
	Min	0.00	0.03	0.01	0.00	0.03	0.02	0.00	0.02	0.01	0.00	0.03	0.01
	Median	0.02	0.06	0.04	0.03	0.04	0.03	0.02	0.04	0.02	0.01	0.05	0.02
	Max	3.13	0.31	0.07	0.94	0.07	0.19	0.22	0.24	3.38	0.06	0.18	3.96
Soluble Na^{+} ($cmol_c\ kg^{-1}$)	Mean	6.80	0.18	0.96	1.02	0.28	1.82	0.36	0.66	2.01	0.19	0.81	2.22
	Std	10.16	0.15	1.73	1.75	0.34	1.90	0.85	0.63	2.30	0.31	0.70	2.41
	Min	0.00	0.00	0.01	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.01
	Median	0.05	0.18	0.06	0.08	0.22	1.91	0.06	0.50	0.28	0.06	0.72	2.21
	Max	28.59	0.40	4.05	4.65	0.98	4.53	3.14	1.63	4.58	1.12	2.04	4.43

Appendix C: Soil physical characterisation of the study sites

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
N		13	8	9	13	7	9	13	8	9	12	8	6
Clay (%)	Mean	31.28	45.91	23.83	28.69	47.42	23.42	35.48	49.03	28.21	40.56	53.18	29.93
	Std	17.33	13.49	13.05	17.03	12.93	9.15	16.70	12.60	11.89	14.53	12.86	15.05
	Min	8.80	23.59	9.83	7.48	24.64	8.30	12.82	22.46	11.24	21.14	26.58	11.97
	Median	35.41	46.03	22.00	36.16	49.70	21.16	39.00	53.41	28.15	42.05	57.54	28.39
	Max	57.12	61.95	43.99	51.77	60.53	35.25	69.51	61.89	47.62	66.19	65.95	47.63
Coarse sand (%)	Mean	4.10	2.40	5.16	4.21	2.15	2.87	4.77	2.07	4.09	3.45	1.66	4.93
	Std	3.89	1.51	4.45	4.53	1.15	2.26	5.13	1.12	2.97	3.98	0.68	4.12
	Min	0.20	1.10	0.13	0.46	1.00	0.07	0.27	1.10	0.73	0.53	1.15	1.06
	Median	2.76	1.88	4.19	1.85	1.70	1.73	3.39	1.60	4.45	2.05	1.37	4.51
	Max	11.80	5.00	13.21	15.41	4.00	6.45	17.99	4.50	10.04	12.77	3.00	11.36
Medium sand (%)	Mean	5.01	3.28	5.88	6.18	3.16	6.34	5.57	2.99	6.44	4.03	2.71	4.86
	Std	3.99	2.14	2.19	5.02	1.88	1.91	4.49	1.05	2.73	3.27	1.11	2.61
	Min	0.99	1.60	1.39	1.06	1.30	3.79	1.00	1.70	2.80	1.06	1.40	2.66
	Median	4.90	2.43	6.04	4.55	2.35	6.17	3.72	2.80	6.31	2.63	2.42	3.65
	Max	15.79	8.00	8.79	17.93	6.50	10.50	14.47	5.05	11.52	12.84	4.50	8.57
Fine sand (%)	Mean	22.65	7.89	24.97	24.15	8.18	28.25	19.18	7.62	26.60	17.89	6.97	21.77
	Std	17.41	7.85	8.59	15.62	7.49	4.38	12.89	7.72	10.63	8.68	6.72	10.33
	Min	5.26	3.30	9.49	6.04	2.50	23.49	4.15	2.50	8.93	3.74	1.90	9.17
	Median	13.93	5.30	27.83	21.85	6.00	26.99	20.99	5.97	23.84	19.84	4.92	20.66
	Max	64.14	26.70	35.95	57.68	24.65	37.27	46.66	26.35	40.91	32.53	23.20	35.32

		Topsoil			A horizon			B1 horizon			B2 horizon		
		Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality	Mortality sites	Sites without mortality	Sites without mortality
Group		Old	New	Old	Old	New	Old	Old	New	Old	Old	New	Old
Very fine sand (%)	Mean	13.23	7.34	12.45	15.25	7.29	14.90	11.15	7.02	12.2	11.42	7.28	12.94
	Std	5.96	5.99	2.90	7.22	6.08	2.89	5.01	6.08	3.96	4.21	6.65	5.75
	Min	7.69	3.00	7.36	7.62	2.15	11.27	4.47	4.10	6.79	4.92	2.00	6.78
	Median	11.40	4.87	13.38	13.98	5.30	15.61	8.50	4.80	12.77	11.94	5.73	12.58
	Max	27.97	21.20	16.67	35.04	20.25	20.00	20.47	22.00	18.80	19.54	23.00	21.82
Coarse silt (%)	Mean	7.80	12.75	7.15	5.35	11.95	6.08	7.06	12.18	6.61	7.48	10.60	5.47
	Std	4.86	4.32	2.21	2.14	4.53	1.96	4.09	4.09	1.65	4.92	1.61	2.68
	Min	1.84	7.25	3.46	0.99	5.61	2.70	0.90	3.96	4.63	1.89	8.26	2.06
	Median	6.88	12.99	8.15	5.26	14.75	7.04	6.07	13.68	5.95	7.04	10.63	4.93
	Max	19.85	20.33	10.12	8.97	16.47	8.26	16.06	15.61	8.94	17.79	13.54	9.08
Electrical resistance Ω	Mean	2292	1331	2451	1188	688	1243	949	593	693	768	540	537
	Std	1386	1652	1201	663	290	370	686	380	277	525	452	229
	Min	300	470	1140	200	295	770	220	240	330	180	205	240
	Median	2240	630	1670	990	710	1130	630	538	720	580	473	595
	Max	4740	5250	4050	2360	1150	1790	2600	1350	1090	1920	1600	870
Electrical conductivity	Mean	21.00	7.56	19.45	148.45	7.33	28.20	36.35	15.73	46.53	22.05	19.67	56.90
	Std	29.35	3.69	20.52	209.73	4.12	29.22	39.34	12.48	55.94	20.47	14.58	64.85
	Min	4.08	1.37	3.51	2.83	1.48	4.06	1.86	4.59	3.29	2.66	1.19	4.33
	Median	10.19	7.04	9.64	16.15	7.72	6.01	22.11	14.10	5.69	11.49	18.75	37.88
	Max	113.70	13.25	56.96	643.50	12.76	82.55	113.70	41.05	160.60	64.53	44.20	165.10

Appendix D: Detailed chemical analytical data

Farm ID	Mortalities	Layer	pH		Resist.	EC	OC	Phosphorous			Soluble				Exchangeable				CEC
								Olsen	H_2O	$CaCl_2$	Ca	Mg	K	Na	Ca	Mg	K	Na	
			H_2O	KCl	(ohm)	(mS m^{-1})		(mg kg^{-1})							($cmol_c$ kg^{-1})				
p013bradlpn	Reported	1	9.18	8.18	1840	37.8	67300	6.5	0.6	0.2	0.08	0.01	0.01	0.32	26.40	6.50	1.40	2.43	9.22
p013bradlpn	Reported	2	9.80	8.29	930	26.5	53000	34.1	0.4	0.1	0.08	0.01	0.01	0.23	25.40	4.17	0.53	0.25	5.48
p013bradlpn	Reported	3	8.72	8.09	550	27.9	72200	10.3	0.5	0.2	0.06	0.01	0.00	0.15	34.10	3.17	3.70	0.83	4.00
p013bradlpn	Reported	4	8.86	8.02	180	18.9	59700	20.9	0.6	0.3	0.03	0.00	0.00	0.10	37.10	2.83	0.40	1.22	5.39
p001brawtkr	Reported	1	6.52	4.50	1520	10.1	20000	40.4	0.4	0.2	0.05	0.00	0.00	0.00	8.90	3.33	1.39	0.18	16.35
p001brawtkr	Reported	2	7.23	4.71	870	2.8	10000	17.8	0.3	0.1	0.03	0.00	0.00	0.00	7.15	2.50	1.89	0.13	12.96
p001brawtkr	Reported	3	7.11	5.16	480	1.9	5400	-1.0	0.3	0.6	0.04	0.00	0.00	0.00	10.25	2.83	1.42	0.30	15.91
p001brawtkr	Reported	4	7.67	6.72	460	2.7	10900	-1.5	0.2	0.1	0.10	0.01	0.00	0.00	29.70	4.17	1.69	0.25	20.00
p002bulwltv	Reported	1	5.44	4.15	2260	4.3	31500	29.4	0.7	0.2	0.07	0.01	0.00	0.02	6.50	2.67	2.71	0.33	22.52
p002bulwltv	Reported	2	6.37	4.65	990	4.8	12600	7.4	0.4	0.2	0.04	0.00	0.00	0.02	10.53	3.67	2.12	0.51	18.52
p002bulwltv	Reported	3	7.31	5.57	560	4.9	7500	47.7	0.6	0.4	0.06	0.01	0.00	0.06	12.16	4.50	1.47	1.27	9.30
p002bulwltv	Reported	4	7.62	5.98	530	9.5	7700	43.2	0.1	0.3	0.08	0.01	0.00	0.06	16.30	4.33	1.39	1.37	20.52
p004bullmrl	Reported	1	10.20	9.60	900	10.2	16700	51.1	11.5	0.6	0.15	0.02	0.01	12.72	18.10	2.67	1.36	6.09	5.13
p004bullmrl	Reported	2	9.37	8.46	770	348.6	15700	17.2	6.7	0.3	0.09	0.03	0.00	4.13	21.30	2.67	1.73	4.87	8.17
p004bullmrl	Reported	3	9.35	8.15	430	103.4	15800	7.0	1.0	0.2	0.06	0.02	0.00	0.43	27.20	5.00	1.58	4.17	12.78
p004bullmrl	Reported	4	9.17	7.83	390	43.7	21100	3.4	1.0	0.2	0.02	0.01	0.00	0.26	24.20	5.67	1.87	3.74	14.00
p009deaqwgg	Reported	1	7.67	6.92	4340	12.4	64300	17.6	0.2	0.5	0.28	0.07	0.00	0.05	32.30	10.83	1.52	1.46	34.61
p009deaqwgg	Reported	2	8.47	7.06	1530	16.2	55500	16.2	1.1	0.3	0.15	0.04	0.00	0.08	37.10	9.83	0.95	1.99	30.26
p009deaqwgg	Reported	3	7.31	6.50	2600	10.3	33400	8.8	0.9	0.4	0.13	0.04	0.00	0.01	23.10	13.83	1.59	0.38	22.00
p009deaqwgg	Reported	4	8.10	7.20	760	12.7	67800	4.9	0.6	0.4	0.11	0.03	0.00	0.05	38.00	8.00	0.61	0.54	22.09
p009deaqwgg	Reported	1	7.17	6.48	3800	8.7	51500	19.2	1.7	0.7	0.13	0.04	0.00	0.01	25.70	1.18	1.99	0.57	31.91
p009deaqwgg	Reported	2	7.92	7.04	1130	9.2	32300	1.9	0.7	0.4	0.14	0.04	0.00	0.05	40.40	13.17	1.17	2.14	13.91
p009deaqwgg	Reported	3	8.09	7.16	950	8.2	25000	0.9	0.4	0.3	0.09	0.03	0.00	0.02	34.00	19.67	1.90	0.49	18.26
p009deaqwgg	Reported	4	8.31	7.29	600	5.8	37400	-0.8	0.6	0.4	0.02	0.01	0.00	0.04	31.60	20.00	1.40	2.01	29.04
p011petmrtn	Reported	1	8.10	7.91	2960	4.6	43500	29.0	0.5	0.4	0.35	3.13	0.02	28.59	34.60	16.83	1.64	0.77	15.39
p011petmrtn	Reported	2	8.04	7.55	2310	397.6	42700	16.3	0.6	0.4	0.26	0.94	0.01	4.65	29.90	13.33	2.99	4.00	22.96
p011petmrtn	Reported	3	8.14	7.60	1390	113.7	33300	6.0	0.8	0.3	0.27	0.22	0.00	3.14	29.00	10.67	2.38	3.13	18.35
p011petmrtn	Reported	4	8.12	7.52	1320	64.5	24400	1.9	0.4	0.5	0.12	0.06	0.00	0.22	24.40	9.67	1.66	1.76	16.26
p010jacrtrv	Reported	1	10.43	9.96	470	113.7	8000	15.3	3.0	0.9	0.01	0.00	0.01	10.65	9.49	2.28	1.15	7.65	8.43
p010jacrtrv	Reported	2	9.60	8.47	410	319.4	8700	4.9	0.8	0.3	0.04	0.01	0.00	0.23	24.90	3.67	0.46	2.53	11.91
p010jacrtrv	Reported	3	8.94	8.05	440	26.7	18200	2.7	0.7	0.9	0.03	0.00	0.00	0.08	29.70	4.17	0.23	0.77	14.43
p008oppdmsh	Reported	1	7.92	7.21	2240	10.0	31200	45.8	0.5	0.7	0.29	0.03	0.00	0.04	36.60	5.00	3.00	0.82	15.30
p008oppdmsh	Reported	2	8.08	7.29	1380	11.8	22300	13.7	0.6	0.5	0.20	0.02	0.00	0.02	38.40	5.33	2.39	0.35	12.35
p008oppdmsh	Reported	3	8.21	7.38	780	5.7	18300	5.2	0.8	0.4	0.11	0.02	0.00	0.01	34.20	5.50	1.52	0.38	14.61
p008oppdmsh	Reported	4	8.39	7.50	570	4.1	18200	2.1	0.4	0.4	0.07	0.01	0.00	0.02	29.70	5.67	1.21	0.58	11.57
p007lucwtrp	Reported	1	10.28	10.00	300	4.1	5100	17.1	3.4	0.5	0.04	0.01	0.10	25.11	6.94	2.00	1.07	2.23	3.83
p007lucwtrp	Reported	2	10.02	9.35	200	643.5	4600	0.9	5.9	0.0	0.06	0.06	0.02	3.39	11.12	3.33	0.88	5.74	4.17
p007lucwtrp	Reported	3	9.77	8.82	220	81.3	10900	-1.2	2.0	0.0	0.07	0.06	0.01	0.38	12.73	4.83	0.87	5.22	5.74
p007lucwtrp	Reported	4	9.64	8.54	400	44.4	12100	-2.4	4.2	0.1	0.05	0.05	0.03	0.36	6.96	6.33	0.97	4.35	8.43

Farm ID	Mortalities	Layer	pH		Resist.	EC	OC	Phosphorous			Soluble				Exchangeable				CEC
								Olsen	H_2O	$CaCl_2$	Ca	Mg	K	Na	Ca	Mg	K	Na	
			H_2O	KCl	(ohm)	(mS m^{-1})		(mg kg^{-1})							($cmol_c$ kg^{-1})				
p005petbrkp	Reported	1	8.20	7.79	4740	22.0	76700	43.3	2.8	0.1	0.29	0.90	0.02	10.87	32.30	13.00	2.23	8.26	24.09
p005petbrkp	Reported	2	8.24	7.55	2360	131.1	48400	25.4	1.5	0.1	0.14	0.07	0.00	0.44	31.90	8.17	1.09	2.37	18.96
p005petbrkp	Reported	3	8.30	7.65	1730	56.6	39200	12.8	0.6	0.0	0.12	0.05	0.00	0.30	32.90	7.50	0.92	1.85	19.22
p005petbrkp	Reported	4	8.34	7.59	1920	39.4	35700	5.2	0.6	0.1	0.27	0.05	0.00	1.12	47.50	23.96	0.96	3.53	31.30
p012redbgnv	Reported	1	7.28	5.85	1990	20.7	18700	22.0	0.6	0.1	0.24	0.02	0.00	0.02	37.75	21.04	3.84	0.42	40.00
p012redbgnv	Reported	2	7.01	5.82	780	4.0	4100	10.7	0.6	0.1	1.06	0.06	0.01	0.02	34.38	12.29	2.24	0.18	36.52
p012redbgnv	Reported	3	7.72	6.69	1580	9.8	14100	4.5	0.5	0.1	0.83	0.04	0.00	0.02	49.75	10.42	1.64	0.14	22.61
p012redbgnv	Reported	4	6.96	5.50	1490	10.3	12500	17.4	0.4	0.0	0.18	0.01	0.00	0.02	38.00	14.58	2.75	0.15	36.52
p014blo7dms	Reported	1	7.36	6.31	2420	14.4	27100	4.5	0.2	0.1	0.28	0.03	0.00	0.03	38.50	14.79	0.98	0.32	32.17
p014blo7dms	Reported	2	7.28	6.18	1790	14.4	20000	-1.1	0.3	0.1	0.21	0.02	0.00	0.03	33.38	13.13	0.86	0.33	16.17
p014blo7dms	Reported	3	7.54	6.03	630	22.1	7900	0.7	0.3	0.1	0.13	0.01	0.00	0.02	29.38	13.33	0.60	0.30	34.78
p014blo7dms	Reported	4	7.80	6.30	590	8.6	6500	-2.3	0.2	0.0	0.12	0.01	0.00	0.02	3.53	19.38	0.44	0.32	37.39
p003bulmgkr	Not reported	1	7.41	6.50	3610	7.1	40300	28.8	1.3	0.1	0.34	0.02	0.01	0.17	48.63	12.29	4.41	2.26	26.96
p003bulmgkr	Not reported	2	8.08	7.23	1600	4.8	16900	11.3	0.3	0.1	0.83	0.07	0.00	1.91	49.63	15.21	2.37	3.45	26.96
p003bulmgkr	Not reported	3	8.70	7.65	660	4.8	21000	-3.0	0.3	0.1	0.23	0.01	0.00	0.28	56.63	12.29	1.29	3.05	23.48
p006bftddmm	Not reported	1	8.21	7.08	3240	57.0	30600	17.9	1.1	0.1	0.73	0.06	0.01	3.97	45.00	23.75	6.20	15.43	41.74
p006bftddmm	Not reported	2	9.34	7.91	1080	52.6	18700	5.0	2.4	0.1	0.29	0.03	0.00	4.53	40.50	14.58	4.03	30.87	35.65
p006bftddmm	Not reported	3	10.04	8.84	330	63.5	17600	0.7	1.6	0.1	0.12	0.01	0.01	4.51	43.50	6.67	2.56	22.61	15.83
p006bftddmm	Not reported	4	9.98	8.91	240	69.4	38400	-1.7	0.7	0.1	0.10	0.01	0.01	4.43	40.25	12.08	2.05	19.57	1.04
p006bftddmm	Not reported	1	9.08	7.75	1500	51.9	20600	8.8	2.5	0.1	0.26	0.02	0.01	4.05	40.00	11.46	4.41	26.09	37.39
p006bftddmm	Not reported	2	9.46	8.21	770	82.6	25500	4.3	2.1	0.1	0.23	0.02	0.01	4.46	39.88	8.75	3.84	43.04	38.26
p006bftddmm	Not reported	3	9.38	8.33	380	90.9	66000	1.3	0.9	0.1	0.17	0.02	0.01	4.58	32.63	8.75	1.73	17.39	29.57
p015kimgrsp	Not reported	1	5.75	4.86	1140	3.5	10000	22.9	0.4	0.1	0.20	0.01	0.00	0.03	14.21	7.29	2.49	0.20	18.00
p015kimgrsp	Not reported	2	6.28	5.05	780	4.1	7700	13.7	0.4	0.1	0.20	0.02	0.00	0.02	17.51	8.96	2.05	0.23	24.35
p015kimgrsp	Not reported	3	7.38	6.01	390	3.3	4800	5.0	0.4	0.1	0.14	0.01	0.00	0.02	33.63	13.96	2.43	0.17	40.87
p015kimgrsp	Not reported	1	7.70	6.81	1410	5.8	16300	86.6	0.3	0.1	0.54	0.04	0.01	0.01	40.13	13.75	0.13	0.12	32.17
p015kimgrsp	Not reported	2	7.79	6.85	1110	6.0	15000	20.5	0.9	0.1	0.46	0.03	0.00	0.01	37.13	10.63	3.32	0.10	28.70
p015kimgrsp	Not reported	3	7.95	7.12	990	5.7	11700	10.1	0.3	0.1	0.49	0.04	0.00	0.02	52.50	11.88	3.45	0.11	31.30
p015kimgrsp	Not reported	4	8.16	7.41	870	5.1	18400	1.0	0.3	0.2	0.51	0.03	0.00	0.02	57.13	11.25	2.88	0.25	26.96
p015kimgrsp	Not reported	1	7.76	7.23	4050	11.2	63900	32.5	1.1	0.2	1.49	0.07	0.01	0.05	61.13	7.71	2.69	0.14	20.87
p015kimgrsp	Not reported	2	8.04	7.41	1660	5.7	22400	21.0	0.4	0.1	0.83	0.03	0.01	0.01	52.00	5.63	1.92	0.09	17.39
p015kimgrsp	Not reported	3	8.22	7.57	810	5.7	20000	8.2	0.6	0.1	0.38	0.02	0.00	0.02	61.50	7.08	3.25	0.18	15.30
p015kimgrsp	Not reported	4	8.43	7.80	310	6.4	34200	2.2	0.4	0.3	0.34	0.02	0.00	0.02	56.63	6.67	1.27	0.16	10.52
p015kimgrsp	Not reported	1	7.99	7.29	3850	7.4	52700	32.9	0.8	0.2	0.55	0.03	0.01	0.03	50.00	6.88	2.88	0.13	34.78
p015kimgrsp	Not reported	2	8.05	7.33	1790	5.9	19700	17.5	0.2	0.1	0.36	0.02	0.01	0.02	50.75	6.46	2.94	0.12	17.74
p015kimgrsp	Not reported	3	8.14	7.54	1090	4.9	23100	6.2	0.4	0.1	0.33	0.01	0.00	0.01	55.50	5.42	4.08	0.08	20.87
p015kimgrsp	Not reported	4	8.34	7.78	610	4.3	26700	0.5	0.2	0.1	0.26	0.01	0.00	0.01	61.38	5.21	2.01	0.07	8.96
p015kimgrsp	Not reported	1	8.49	7.53	1590	21.6	25500	12.4	0.5	0.1	0.68	0.04	0.01	0.25	70.50	13.13	5.37	3.23	38.26
p015kimgrsp	Not reported	2	8.82	7.58	1130	43.0	23800	6.8	2.5	0.1	0.49	0.03	0.01	2.57	61.00	5.00	5.95	14.67	40.00
p015kimgrsp	Not reported	3	9.30	8.03	870	79.4	31100	3.9	0.7	0.2	0.25	0.02	0.01	4.30	58.13	11.88	5.69	36.30	47.83
p015kimgrsp	Not reported	4	9.42	8.13	600	91.1	30900	2.6	0.4	0.1	0.31	0.03	0.02	4.40	49.88	10.42	7.29	46.20	52.17
p015kimgrsp	Not reported	1	8.27	7.70	1670	9.6	38600	14.0	0.2	0.1	0.61	0.04	0.01	0.06	58.88	9.38	1.66	0.54	20.87
p015kimgrsp	Not reported	2	8.20	7.77	1270	49.2	42200	10.6	0.2	0.1	1.57	0.19	0.03	2.85	53.38	14.58	2.88	2.52	17.39
p015kimgrsp	Not reported	3	8.15	8.01	720	160.6	67100	4.9	0.1	0.1	1.49	3.38	0.12	4.33	39.75	21.67	3.77	5.54	16.52
p015kimgrsp	Not reported	4	8.29	8.12	590	165.1	69100	1.6	0.5	0.1	0.60	3.96	0.14	4.41	35.63	23.96	3.58	3.32	14.70

Farm ID	Mortalities	Layer	pH		Resist.	EC	OC	Phosphorous			Soluble				Exchangeable				CEC
								Olsen	H_2O	$CaCl_2$	Ca	Mg	K	Na	Ca	Mg	K	Na	
			H_2O	KCl	(ohm)	(mS m^{-1})		(mg kg^{-1})							($cmol_c$ kg^{-1})				
321hoppplr	Not reported	1	7.66	7.31	730	13.3	60450	57.7	4.2	2.0	0.23	0.31	0.07	0.23	55.70	18.69	1.71	0.29	4.46
321hoppplr	Not reported	2	8.24	7.63	710	7.7	14363	26.3	1.2	0.2	0.13	0.07	0.03	0.22	51.89	15.31	1.39	0.48	1.63
321hoppplr	Not reported	3	8.61	7.91	740	14.6	7725	16.9	0.9	0.1	0.09	0.06	0.03	0.53	52.50	18.78	1.07	1.11	1.10
321hoppplr	Not reported	4	9.07	7.92	475	21.0	6173	16.5	1.1	0.1	0.08	0.05	0.03	0.87	46.87	17.53	0.69	0.70	1.23
222phibthl	Not reported	1	7.99	7.20	480	11.0	30975	40.8	3.4	0.7	0.13	0.06	0.08	0.40	55.31	15.77	1.89	1.08	3.43
222phibthl	Not reported	2	8.53	7.34	375	12.1	19530	15.4	1.2	0.3	0.10	0.04	0.05	0.40	39.40	12.30	1.83	1.48	4.23
222phibthl	Not reported	3	8.57	7.34	425	14.8	16418	7.7	0.9	0.1	0.09	0.03	0.09	0.48	46.80	15.60	1.73	1.65	4.50
222phibthl	Not reported	4	8.77	7.52	475	16.5	7628	4.4	0.4	0.4	0.13	0.05	0.03	0.34	41.78	9.09	0.60	1.71	0.80
259edemdws	Not reported	1	6.31	5.32	600	7.4	33435	44.0	1.0	0.3	0.26	0.13	0.04	0.13	17.22	9.19	2.44	0.32	4.80
259edemdws	Not reported	2	6.84	5.62	725	4.0	8828	55.5	1.9	0.2	0.14	0.05	0.08	0.07	17.07	9.14	1.82	0.43	2.03
259edemdws	Not reported	3	7.06	5.88	650	4.6	8153	51.4	2.0	0.3	0.16	0.05	0.09	0.08	19.78	8.60	1.40	0.44	3.23
259edemdws	Not reported	4	7.72	6.72	470	6.2	8648	37.3	1.4	0.5	0.24	0.08	0.03	0.08	41.32	9.29	1.06	0.39	5.30
211bosxcls	Not reported	1	7.83	6.92	660	6.7	13095	2.4	0.4	0.2	0.43	0.10	0.02	0.04	19.64	7.48	1.95	0.23	3.15
211bosxcls	Not reported	2	8.27	6.99	680	12.8	11115	1.7	0.3	0.1	0.22	0.06	0.03	0.28	30.72	10.93	1.49	0.78	1.72
211bosxcls	Not reported	3	8.36	7.54	250	41.0	6165	0.4	0.2	1.7	0.34	0.24	0.06	1.46	42.23	21.50	1.30	1.59	0.91
211bosxcls	Not reported	4	8.48	7.59	235	35.9	3698	0.5	0.2	0.2	0.29	0.18	0.06	1.29	50.85	20.89	0.82	1.74	0.88
284petkrfs	Not reported	1	5.51	3.76	1900	1.4	15840	40.5	0.5	0.1	0.18	0.04	0.03	0.00	14.38	8.65	1.88	0.21	3.18
284petkrfs	Not reported	2	5.62	4.06	1150	1.5	6240	23.8	0.4	0.1	0.20	0.04	0.02	0.02	10.05	6.52	1.02	0.21	1.77
284petkrfs	Not reported	3	5.87	4.39	1350	4.7	4290	12.6	0.5	0.6	0.18	0.03	0.04	0.00	8.61	5.27	0.79	0.19	1.30
284petkrfs	Not reported	4	6.06	4.55	1600	1.2	2798	19.1	0.6	0.6	0.16	0.03	0.02	0.00	7.88	4.75	0.80	0.22	1.16
140troklkn	Not reported	1	7.82	6.13	470	6.6	8903	50.8	2.8	0.5	0.20	0.03	0.05	0.29	13.82	6.22	1.15	1.38	1.65
140troklkn	Not reported	2	9.30	7.41	295	8.0	2768	10.8	7.0	0.9	0.20	0.03	0.02	0.98	25.03	6.75	0.77	1.29	1.12
140troklkn	Not reported	3	9.48	7.62	240	26.1	367	9.9	6.9	3.1	0.19	0.02	0.03	0.95	20.72	6.11	0.70	1.49	1.17
140troklkn	Not reported	4	9.66	7.85	255	21.9	517	10.4	9.5	2.0	0.19	0.03	0.08	1.33	20.81	6.77	0.63	1.60	1.14
131smibssv	Not reported	1	7.13	6.14	5250	4.8	17790	19.1	6.7	0.6	0.28	0.06	0.02	0.03	12.94	5.40	1.87	0.27	0.92
131smibssv	Not reported	3	8.05	7.01	820	5.2	7973	14.2	1.3	0.9	0.27	0.05	0.04	0.06	14.94	6.00	1.21	0.33	0.85
131smibssv	Not reported	4	8.29	7.03	805	6.3	4658	7.5	0.7	0.2	0.24	0.04	0.02	0.14	13.83	10.33	1.09	0.74	1.09
131smibssv	Not reported	5	8.96	7.48	605	10.5	3653	3.5	0.9	0.5	0.13	0.04	0.32	0.57	26.09	24.35	1.33	4.43	27.06
131smibssv	Not reported	1	7.95	6.93	555	9.3	28110	4.4	0.8	0.5	0.16	0.03	0.15	0.30	26.23	9.08	1.41	3.60	32.77
131smibssv	Not reported	2	8.96	7.25	285	13.6	2925	8.2	1.0	0.5	0.25	0.06	0.12	1.63	38.28	10.25	0.75	5.78	30.76
131smibssv	Not reported	3	8.66	7.12	205	44.2	2843	3.1	0.8	0.3	0.30	0.08	0.20	2.04	34.77	9.28	0.72	6.83	38.93

Appendix E: Detailed physical analytical data

Farm ID	Layer	Coarse sand	Medium sand	Fine sand	Very fine sand	Total sand	Coarse silt	Fine Silt	Total silt	Clay	Total
						(%)					
p013bradlpn	1	3.1	5.6	24.2	28.0	60.9	10.9	10.6	21.6	10.2	92.6
p013bradlpn	2	1.9	4.4	30.4	35.0	71.7	6.1	7.2	13.3	10.5	95.6
p013bradlpn	3	1.7	3.5	15.3	20.5	41.0	6.6	10.7	17.3	40.1	98.5
p013bradlpn	4	0.5	1.5	20.9	19.5	42.5	8.5	17.2	25.7	28.5	96.8
p001brawtkr	1	1.0	1.1	13.4	11.4	26.9	5.0	15.0	20.0	57.1	104.0
p001brawtkr	2	0.6	1.1	23.9	19.9	45.5	4.2	8.9	13.1	38.2	96.8
p001brawtkr	3	0.3	1.1	26.9	7.9	36.3	13.2	6.1	19.3	39.0	94.6
p001brawtkr	4	2.4	2.7	18.0	14.0	37.0	8.5	8.8	17.2	50.1	104.4
p002bulwltv	1	2.8	2.9	12.3	11.3	29.3	6.4	12.9	19.3	50.1	98.7
p002bulwltv	2	0.7	2.4	19.6	14.7	37.4	5.4	8.6	14.0	44.8	96.2
p002bulwltv	3	0.3	2.7	21.0	15.0	38.9	4.2	9.1	13.3	43.7	95.9
p002bulwltv	4	0.6	2.5	19.9	11.7	34.7	3.1	9.4	12.5	48.5	95.7
p004bullmrl	1	1.4	5.2	46.6	21.8	75.0	4.7	2.6	7.3	14.0	96.3
p004bullmrl	2	8.7	6.7	44.2	14.0	73.6	4.0	4.1	8.1	15.8	97.5
p004bullmrl	3	7.4	3.7	33.4	16.3	60.8	3.7	8.8	12.5	25.3	98.6
p004bullmrl	4	0.8	2.6	19.7	11.2	34.3	12.5	7.7	20.2	45.7	100.2
p009deaqwgg	1	11.4	4.9	19.0	10.6	45.8	1.8	15.3	17.1	41.9	104.9
p009deaqwgg	2	2.9	3.6	11.6	10.5	28.7	5.4	17.8	23.2	46.4	98.3
p009deaqwgg	3	5.6	8.1	4.9	4.5	23.0	7.4	23.8	31.3	47.8	102.0
p009deaqwgg	4	10.2	2.3	4.2	5.2	21.9	3.9	13.1	17.0	58.0	96.9
p009deaqwgg	1	3.4	3.5	6.9	8.1	21.9	6.9	25.1	32.0	47.4	101.3
p009deaqwgg	2	0.7	1.8	9.8	11.8	24.2	4.3	19.5	23.8	51.8	99.8
p009deaqwgg	3	3.2	2.5	4.1	5.2	15.1	0.9	18.9	19.8	69.5	104.4
p009deaqwgg	4	3.3	2.5	3.7	4.9	14.5	2.6	17.3	19.9	66.2	100.6
p011petmrtn	1	0.2	1.0	5.3	8.3	14.7	9.6	32.7	42.4	46.3	103.4
p011petmrtn	2	0.5	1.1	6.7	8.6	16.8	5.3	28.9	34.2	48.7	99.7
p011petmrtn	3	0.7	1.5	8.1	7.7	18.0	6.1	22.7	28.8	49.8	96.6
p011petmrtn	4	1.5	3.5	18.9	13.4	37.3	5.6	16.3	21.9	44.6	103.8
p010jacrtrv	1	6.5	5.2	38.4	8.2	58.3	19.9	5.0	24.8	8.8	91.9
p010jacrtrv	2	8.8	11.4	40.6	18.1	78.9	4.8	3.9	8.7	8.9	96.5
p010jacrtrv	3	11.8	12.6	27.0	14.8	66.3	4.3	5.2	9.5	21.8	97.6
p008oppdmsh	1	11.8	5.1	13.3	7.7	37.9	4.0	22.7	26.7	35.4	100.0
p008oppdmsh	2	6.9	5.1	15.8	7.6	35.4	3.9	19.8	23.7	36.2	95.3
p008oppdmsh	3	1.5	1.0	8.4	8.5	19.3	5.8	23.1	28.9	49.7	97.9
p008oppdmsh	4	1.7	3.8	20.5	10.7	36.8	3.7	14.0	17.7	39.5	93.9
p007lucwtrp	1	1.7	9.8	64.1	12.3	88.0	2.1	2.7	4.8	10.3	103.0
p007lucwtrp	2	1.6	12.1	57.7	12.7	84.0	1.0	2.5	3.5	7.5	95.1
p007lucwtrp	3	3.4	10.3	46.7	8.0	68.4	7.8	5.3	13.1	12.8	94.3
p007lucwtrp	4	2.6	6.7	32.5	12.1	54.0	1.9	17.9	19.8	23.1	96.8

Farm ID	Layer	Coarse sand	Medium sand	Fine sand	Very fine sand	Total sand	Coarse silt	Fine Silt	Total silt	Clay	Total
						(%)					
p005petbrkp	1	1.9	2.9	13.9	14.7	33.4	8.4	21.7	30.1	30.6	94.1
p005petbrkp	2	4.4	4.5	25.9	20.8	55.7	9.0	13.5	22.5	13.6	91.8
p005petbrkp	3	4.7	6.3	26.3	15.6	52.9	10.3	12.3	22.6	18.6	94.1
p005petbrkp	4	4.3	6.4	27.9	6.8	45.5	17.8	8.7	26.5	21.1	93.1
p012redbgnv	1	1.1	2.1	10.2	13.2	26.6	12.2	22.2	34.5	38.3	99.3
p012redbgnv	2	1.7	8.1	6.0	10.2	26.1	8.0	23.8	31.7	36.3	94.1
p012redbgnv	3	3.4	4.6	6.0	7.4	21.4	16.1	31.8	47.8	26.7	95.9
p012redbgnv	4	0.5	1.1	8.1	12.4	22.1	12.3	24.8	37.1	39.1	98.3
p014blo7dms	1	7.0	15.8	26.9	16.5	66.3	9.4	8.7	18.0	16.3	100.6
p014blo7dms	2	15.4	17.9	21.8	14.1	69.3	8.3	8.2	16.5	14.3	100.1
p014blo7dms	3	18.0	14.5	21.2	13.6	67.3	5.4	5.6	11.1	16.5	94.8
p014blo7dms	4	12.8	12.8	20.1	15.2	60.9	9.3	6.8	16.1	22.3	99.3
p003bulmgkr	1	1.1	5.7	23.9	14.1	44.9	6.2	14.7	20.9	29.9	95.7
p003bulmgkr	2	1.1	5.4	32.0	20.0	58.6	7.7	10.2	17.9	23.7	100.2
p003bulmgkr	3	2.9	7.5	33.6	18.8	62.8	5.3	8.4	13.8	22.2	98.8
p006bftddmm	1	0.1	1.4	9.5	11.3	22.4	8.2	19.8	28.0	44.0	94.4
p006bftddmm	2	1.0	3.8	26.4	16.2	47.4	5.2	10.3	15.5	34.7	97.7
p006bftddmm	3	0.7	3.9	40.9	15.8	61.3	6.0	8.9	14.9	23.0	99.2
p006bftddmm	4	1.1	2.9	23.3	16.2	43.5	9.1	13.1	22.2	29.6	95.3
p006bftddmm	1	0.9	3.8	16.4	7.4	28.5	5.1	20.8	25.9	42.4	96.7
p006bftddmm	2	0.1	6.7	23.9	11.3	41.9	4.8	13.3	18.1	35.2	95.3
p006bftddmm	3	10.0	5.3	16.2	10.9	42.5	5.4	13.4	18.8	37.4	98.7
p015kimgrsp	1	4.2	8.8	35.9	13.4	62.3	3.5	8.2	11.6	22.0	96.0
p015kimgrsp	2	4.3	7.5	27.0	12.9	51.6	2.7	8.7	11.4	32.2	95.2
p015kimgrsp	3	5.8	8.1	22.2	7.0	43.1	8.9	8.9	17.8	37.9	98.8
p015kimgrsp	1	8.8	6.0	27.8	16.7	59.4	5.4	20.2	25.6	14.5	99.5
p015kimgrsp	2	1.7	6.5	27.3	17.2	52.7	7.2	16.1	23.3	19.5	95.4
p015kimgrsp	3	1.4	4.2	21.8	12.8	40.2	8.1	20.1	28.2	32.6	100.9
p015kimgrsp	4	2.3	3.8	18.0	14.7	38.9	5.6	22.1	27.7	27.2	93.8
p015kimgrsp	1	5.9	7.0	32.5	14.8	60.2	8.6	11.4	20.0	11.6	91.8
p015kimgrsp	2	3.6	6.2	30.7	16.4	56.9	8.3	14.5	22.8	15.0	94.7
p015kimgrsp	3	4.4	6.3	34.4	13.8	59.0	8.0	10.7	18.7	13.9	91.5
p015kimgrsp	4	7.0	7.8	31.7	21.8	68.3	4.3	11.2	15.5	12.0	95.7
p015kimgrsp	1	9.0	7.9	31.4	11.8	60.1	9.2	15.8	25.0	9.8	94.9
p015kimgrsp	2	5.8	10.5	37.3	15.6	69.2	4.0	12.6	16.5	8.3	94.0
p015kimgrsp	3	5.8	8.4	37.5	10.0	61.7	8.2	10.5	18.6	11.2	91.6
p015kimgrsp	4	6.7	8.6	35.3	10.4	61.0	8.1	12.0	20.0	16.0	97.1
p015kimgrsp	1	3.2	6.0	28.4	13.6	51.2	8.1	19.1	27.2	12.7	91.1
p015kimgrsp	2	1.7	4.6	26.2	12.4	45.0	7.0	19.7	26.8	21.0	92.8
p015kimgrsp	3	1.1	11.5	23.8	13.9	50.3	5.1	14.1	19.2	28.1	97.6
p015kimgrsp	4	1.1	3.5	13.1	7.6	25.3	2.1	18.8	20.8	47.1	93.3
p015kimgrsp	1	13.2	6.2	18.8	9.0	47.3	10.1	16.1	26.2	27.6	101.1
p015kimgrsp	2	6.5	5.9	23.5	12.1	48.0	7.9	14.6	22.5	21.2	91.6
p015kimgrsp	3	4.7	2.8	8.9	6.8	23.2	4.6	18.9	23.5	47.6	94.3
p015kimgrsp	4	11.4	2.7	9.2	6.8	30.0	3.7	14.5	18.2	47.6	95.8

Farm ID	Layer	Coarse sand	Medium sand	Fine sand	Very fine sand	Total sand	Coarse silt	Fine Silt	Total silt	Clay	Total
						(%)					
321hoppplr	1	4.5	4.7	8.1	10.0	27.3	12.7	7.9	20.6	33.6	81.5
321hoppplr	2	3.5	4.8	7.5	9.0	24.8	14.8	21.9	36.7	39.9	101.3
321hoppplr	3	2.9	3.6	6.1	6.1	18.7	4.0	33.4	37.3	44.1	100.1
321hoppplr	4	2.3	4.3	6.0	5.8	18.5	13.5	28.6	42.1	41.8	102.3
222phibthl	1	2.3	3.1	6.5	5.1	17.0	20.3	25.2	45.6	40.5	103.0
222phibthl	2	2.0	3.5	7.2	5.3	18.0	15.2	24.7	40.0	41.6	99.6
222phibthl	3	2.0	2.3	5.8	4.6	14.8	14.9	22.5	37.5	55.4	107.6
222phibthl	4	1.8	2.5	6.6	5.1	16.0	10.2	19.9	30.2	58.1	104.2
259edemdws	1	2.1	2.7	7.5	3.0	15.2	16.5	25.2	41.7	40.9	97.8
259edemdws	2	1.7	2.3	6.0	2.1	12.2	8.9	27.2	36.1	49.7	98.0
259edemdws	3	1.6	3.5	6.6	4.1	15.9	15.1	27.1	42.2	42.9	100.9
259edemdws	4	1.2	2.5	5.0	2.3	11.1	9.1	16.8	25.9	62.3	99.2
211bosxcls	1	5.0	8.0	26.7	21.2	60.9	7.3	10.0	17.2	23.6	101.7
211bosxcls	2	4.0	6.5	24.7	20.3	55.4	7.3	13.4	20.7	24.6	100.7
211bosxcls	3	4.5	5.1	26.4	22.0	57.9	8.1	11.6	19.7	22.5	100.1
211bosxcls	4	3.0	4.5	23.2	23.0	53.7	10.0	14.7	24.7	26.6	105.0
284petkrfs	1	1.7	2.2	3.3	4.1	11.3	13.3	21.5	34.8	51.2	97.3
284petkrfs	2	1.6	2.0	5.8	5.7	15.1	5.6	21.1	26.8	60.5	102.4
284petkrfs	3	1.6	3.1	6.1	4.5	15.4	15.6	17.1	32.7	51.7	99.8
284petkrfs	4	1.5	2.2	4.8	6.7	15.2	11.0	16.2	27.2	58.8	101.2
140troklkn	1	1.1	1.8	3.3	4.2	10.4	8.1	22.6	30.8	58.9	100.1
140troklkn	2	1.0	1.3	2.5	4.1	8.9	16.5	21.3	37.7	57.0	103.6
140troklkn	3	1.1	1.7	3.2	4.8	10.8	13.3	17.9	31.2	61.9	103.9
140troklkn	4	1.2	1.4	1.9	2.0	6.5	11.4	29.4	40.8	56.9	104.2
131smibssv	1	1.2	1.6	3.6	6.5	12.9	13.6	11.6	25.1	61.9	99.9
131smibssv	3	1.2	1.5	4.5	4.8	12.0	11.4	20.0	31.4	58.4	101.8
131smibssv	4	1.3	2.5	2.5	4.8	11.2	14.1	16.8	30.9	55.1	97.2
131smibssv	5	1.2	2.4	4.8	7.7	16.1	11.2	17.3	28.5	55.0	99.5
131smibssv	1	1.4	2.2	4.1	4.6	12.4	10.2	15.8	26.0	56.6	95.0
131smibssv	2	1.5	2.2	4.2	5.3	13.2	12.4	17.6	30.1	58.7	101.9
131smibssv	3	1.1	1.9	3.4	5.6	12.1	8.3	17.5	25.8	65.9	103.8

Appendix F: Detailed major element (XRF) analytical data

Farm ID	Layer	Al_2O_3	CaO	Fe_2O_3	K_2O	MgO	MnO	P_2O_5	SiO_2	TiO_2	Na_2O	LOI
						(%)						
p013bradlpn	1	3.5	17.6	1.6	1.0	2.6	0.0	0.0	51.6	0.3	0.6	21.8
p001brawtkr	1	13.0	0.4	6.1	2.1	0.8	0.1	0.1	66.1	0.7	0.3	11.3
p002bulwltv	1	12.2	0.4	5.5	1.8	0.9	0.0	0.1	67.4	0.6	0.4	12.6
p004bullmrl	1	3.1	3.1	2.3	0.9	1.0	0.0	0.1	82.6	0.3	1.4	6.6
p009deaqwgg	1	7.0	6.0	4.7	0.9	2.0	0.0	0.1	57.7	0.6	0.3	21.8
p009deaqwgg	1	9.8	1.6	5.3	1.6	2.0	0.1	0.1	60.2	0.6	0.4	19.3
p011petmrtn	1	8.4	5.8	4.4	1.8	6.3	0.1	0.1	49.7	0.4	1.6	22.4
p010jacrtrv	1	7.0	4.1	5.1	1.0	2.4	0.1	0.0	74.8	0.7	2.0	4.4
p008oppdmsh	1	11.6	4.1	6.3	2.2	3.0	0.1	0.2	58.4	0.5	0.5	14.3
p007lucwtrp	1	2.8	1.4	2.6	0.7	1.3	0.0	0.0	87.8	0.5	2.1	2.9
p005petbrkp	1	3.8	12.3	2.1	0.7	4.2	0.0	0.2	51.7	0.2	1.3	25.3
p012redbgnv	1	13.0	0.9	4.8	2.4	1.1	0.1	0.1	65.0	0.5	1.1	10.1
p014blo7dms	1	13.1	5.4	8.0	1.2	2.8	0.1	0.1	58.7	1.0	1.4	9.9
p003bulmgkr	1	11.0	0.8	5.7	1.6	0.8	0.2	0.1	64.3	0.5	0.3	16.0
p006bftddmm	1	13.6	0.9	6.3	2.2	1.8	0.0	0.1	59.2	0.6	0.7	15.8
p006bftddmm	1	13.5	2.0	6.3	2.2	2.2	0.0	0.1	60.4	0.6	1.0	12.1
p015kimgrsp	1	7.3	0.4	4.8	1.4	0.7	0.1	0.1	79.2	0.4	0.4	6.4
p015kimgrsp	1	7.8	0.7	4.5	1.7	1.8	0.1	0.1	75.2	0.5	0.4	8.7
p015kimgrsp	1	5.6	5.5	3.6	1.4	2.4	0.0	0.1	67.4	0.4	0.5	15.0
p015kimgrsp	1	6.3	3.0	3.8	1.4	2.5	0.1	0.2	71.0	0.4	0.5	12.4
p015kimgrsp	1	5.5	6.4	3.3	1.5	4.6	0.1	0.1	67.0	0.3	0.5	13.5
p015kimgrsp	1	5.2	10.2	3.3	1.2	6.1	0.1	0.1	59.0	0.3	0.4	17.2
321hoppplr	1	6.8	10.8	2.6	1.5	4.8	0.1	0.2	37.7	0.2	0.4	34.2
321hoppplr	2	7.8	14.1	3.4	1.4	5.8	0.1	0.1	40.4	0.3	0.4	24.5
321hoppplr	3	8.3	13.9	3.6	1.5	5.8	0.1	0.1	42.2	0.3	0.5	22.6
321hoppplr	4	7.6	14.9	3.3	1.3	5.3	0.1	0.1	42.9	0.3	0.5	22.3
222phibthl	1	11.3	3.4	5.4	1.9	2.1	0.1	0.2	55.9	0.6	0.8	17.9
222phibthl	2	11.6	4.3	5.3	2.1	2.3	0.1	0.1	58.3	0.6	0.8	14.4
222phibthl	3	11.1	5.8	5.2	2.0	2.2	0.1	0.1	56.5	0.6	1.0	14.3
222phibthl	4	8.9	15.6	4.0	1.6	1.8	0.1	0.2	46.0	0.4	0.7	19.8
259edemdws	1	13.0	0.8	5.2	2.5	1.4	0.0	0.1	60.2	0.6	0.9	14.4
259edemdws	2	13.5	1.0	5.7	2.6	1.6	0.1	0.2	66.0	0.7	1.0	8.7
259edemdws	3	13.2	1.7	5.6	2.4	1.5	0.1	0.2	66.1	0.7	1.0	8.1
259edemdws	4	16.1	2.1	6.8	2.9	1.8	0.2	0.2	78.7	0.8	1.0	-10.4
211bosxcls	1	6.8	1.0	4.7	1.2	1.7	0.1	0.1	73.4	0.8	0.5	8.5
211bosxcls	2	6.7	2.0	4.6	1.2	2.8	0.1	0.1	71.0	0.8	0.5	9.5
211bosxcls	3	5.7	6.4	3.9	1.0	4.9	0.0	0.0	62.6	0.7	0.5	14.1
211bosxcls	4	5.4	9.2	3.7	0.8	5.5	0.0	0.0	57.3	0.6	0.4	16.2
284petkrfs	1	12.9	0.9	7.6	1.3	1.3	0.1	0.2	62.3	0.6	0.4	13.2
284petkrfs	2	10.0	1.3	5.3	1.5	1.3	0.1	0.1	71.7	0.6	0.5	8.2
284petkrfs	3	9.5	1.0	6.4	1.2	1.2	0.1	0.1	72.9	0.8	0.5	6.2
284petkrfs	4	9.3	1.0	6.3	1.1	1.2	0.1	0.1	75.5	0.8	0.5	5.5
140troklkn	1	13.6	0.9	4.9	2.5	1.2	0.1	0.1	68.7	0.6	1.4	7.0
140troklkn	2	13.3	2.1	4.9	2.5	1.5	0.1	0.1	69.1	0.6	1.6	5.9
140troklkn	3	14.0	1.5	5.4	3.1	1.7	0.1	0.1	67.3	0.6	1.6	5.4
140troklkn	4	13.7	1.9	5.4	3.1	1.6	0.1	0.1	66.1	0.6	1.7	5.7
131smibssv	1	10.7	1.0	5.5	1.6	1.2	0.1	0.1	72.8	0.6	1.6	7.2
131smibssv	3	10.9	1.1	4.1	1.9	1.1	0.1	0.1	73.7	0.6	1.6	6.0
131smibssv	4	11.9	0.9	4.9	2.1	1.4	0.1	0.1	71.2	0.6	1.2	6.7
131smibssv	5	13.3	3.9	5.6	2.2	2.6	0.1	0.1	60.4	0.6	0.9	10.8
131smibssv	1	9.6	1.2	3.6	1.6	0.9	0.1	0.1	66.3	0.5	1.3	13.6
131smibssv	2	12.3	2.7	4.5	1.9	1.2	0.0	0.0	69.7	0.5	1.4	8.1
131smibssv	3	12.2	2.5	4.5	2.0	1.2	0.0	0.1	70.0	0.5	1.4	8.2

Appendix G: Detailed minor element (XRF) analytical data

Farm ID	Layer	Sc	V	Cr	Co	Ni	Cu	Zn	As	Br	Rb	Sr	Y	Zr	Nb	Ba	Pb	Th	U	Mo
										(mg kg^{-1})										
p013bradlpn	1	24.1	40.1	37.1	3.5	12.3	25.9	15.2	6.1	16.5	30	1188	9.8	166	<1	569	<2	<2	<2	
p001brawtkr	1	13.6	114.3	121.7	13.2	45.0	40.5	67.7	8.3	3.0	105	46	24.6	227	8	953	18.9	8.5	<2	
p002bulwltv	1	10.1	114.0	106.8	12.9	44.5	19.8	62.4	13.6	8.8	99	50	25.0	269	8	869	16.2	5.4	<2	
p004bullmrl	1	<2	47.5	45.3	5.4	16.9	27.2	16.4	6.9	30.0	30	117	8.6	230	1	465	8.4	<2	<2	
p009deaqwgg	1	7.3	140.5	104.6	11.2	34.5	31.1	52.8	2.5	24.2	40	155	11.6	189	3	682	7.4	<2	<2	
p009deaqwgg	1	10.9	93.1	132.1	14.7	41.7	15.8	68.0	8.2	11.6	67	104	18.2	169	5	828	12.7	3.4	<2	
p011petmrtn	1	7.3	102.8	99.5	11.8	35.3	22.2	68.8	7.4	105.9	75	743	16.3	121	<1	652	10.1	<2	7.0	
p010jacrtrv	1	9.7	120.2	176.7	13.4	37.9	40.0	37.6	2.5	12.6	31	104	10.9	293	4	857	7.3	2.1	<2	
p008oppdmsh	1	9.1	105.8	115.5	16.4	42.1	32.9	79.7	8.0	4.5	98	163	18.6	169	6	896	16.8	4.9	<2	
p007lucwtrp	1	2.1	86.0	53.4	6.3	15.2	38.4	12.5	11.3	66.2	23	157	7.5	348	2	673	9.9	<2	<2	
p005petbrkp	1	11.7	243.0	57.2	5.4	18.6	34.0	43.5	12.5	68.8	28	1008	8.3	120	<1	514	<2	<2	14.3	
p012redbgnv	1	7.0	98.6	77.9	16.2	33.8	32.6	63.2	14.4	6.5	115	151	24.9	198	7	1013	17.9	9.3	<2	
p014blo7dms	1	16.8	178.0	129.2	24.4	46.6	20.4	68.0	2.5	10.3	40	120	20.9	183	4	663	13.1	<2	<2	
p003bulmgkr	1	9.5	94.9	101.0	23.9	42.4	24.2	57.1	8.4	41.9	80	66	20.0	206	5	783	14.7	3.2	<2	
p006bftddmm	1	10.1	109.5	127.5	13.3	50.3	36.3	74.3	7.8	19.6	104	112	24.2	196	6	901	17.8	4.5	3.6	
p006bftddmm	1	12.2	108.6	119.0	12.9	49.7	30.0	63.8	6.0	10.7	99	258	23.8	174	5	858	12.9	3.9	<2	
p015kimgrsp	1	6.3	80.9	90.4	11.4	42.5	40.6	42.6	2.5	2.3	54	58	14.8	211	4	635	13.4	4.1	<2	
p015kimgrsp	1	7.2	82.6	94.5	13.5	39.1	28.9	44.6	7.4	3.7	57	64	14.2	233	5	632	11.4	3.2	<2	
p015kimgrsp	1	3.1	67.3	66.1	9.9	30.1	13.8	37.0	5.0	6.2	43	194	12.6	227	3	549	9.5	<2	<2	
p015kimgrsp	1	4.2	75.0	78.0	10.7	32.8	20.6	48.5	11.4	4.8	52	254	14.1	246	3	623	11.9	3.5	<2	
p015kimgrsp	1	<2	57.3	60.5	8.7	28.3	41.3	37.7	2.5	5.6	42	242	10.5	169	2	515	7.1	<2	<2	
p015kimgrsp	1	5.7	65.3	52.9	9.0	24.6	26.9	31.9	6.0	4.8	44	408	11.8	192	<1	512	7.6	<2	2.6	
321hoppplr	1	7.0	63.0	60.5	13.0	30.5	30.0	86.0	18.5	36.0	69	554	18.5	92	6	296	14.0	7.5	5.0	2.0
321hoppplr	2	7.0	84.0	61.0	14.0	31.5	31.0	73.5	18.0	20.0	73	549	19.0	104	7	316	14.0	7.5	4.5	1.0
321hoppplr	3	7.0	88.5	63.5	13.5	32.5	31.5	70.0	21.5	9.0	73	528	19.0	109	7	327	13.0	7.5	5.0	2.0
321hoppplr	4	7.0	85.0	57.5	12.0	28.5	28.5	62.5	19.0	7.0	66	532	18.0	108	7	303	12.5	7.0	4.5	2.0
222phibthl	1	8.0	104.0	116.0	17.5	50.5	47.5	151.0	18.0	31.0	96	224	28.5	201	10	457	20.0	10.0	2.5	1.0
222phibthl	2	7.0	100.5	109.0	19.0	49.5	45.0	133.0	18.5	24.0	94	249	28.0	193	10	446	21.5	9.0	2.0	1.0
222phibthl	3	7.0	93.0	103.5	16.5	46.0	42.0	105.5	19.0	19.0	90	259	27.0	202	10	422	17.5	9.5	1.5	2.0
222phibthl	4	7.0	70.5	69.5	12.0	32.0	27.5	37.0	21.0	17.0	69	284	22.0	156	8	318	14.0	7.0	2.5	2.0
259edemdws	1	23.0	100.5	96.5	18.5	49.0	45.0	230.0	13.5	4.0	133	177	36.5	196	14	536	32.0	14.5	3.5	2.0
259edemdws	2	23.0	103.0	92.5	19.5	48.5	43.5	195.0	16.5	1.0	129	184	36.0	206	14	547	31.0	13.0	3.0	1.0
259edemdws	3	20.0	94.5	88.0	20.0	46.5	39.5	177.0	16.5	1.0	122	197	35.0	221	13	553	29.0	13.0	3.0	2.0
259edemdws	4	13.5	95.0	87.0	21.0	47.0	35.5	138.5	20.5	1.0	111	214	34.5	227	12	548	28.0	12.0	2.5	2.0
211bosxcls	1	12.0	86.0	113.0	17.0	51.0	37.0	64.0	15.0	6.0	48	84	19.0	279	9	255	12.5	4.0	2.0	1.0
211bosxcls	2	8.5	154.5	113.0	17.0	51.5	40.0	53.5	14.0	11.5	47	139	19.0	254	9	265	10.0	5.0	2.0	1.0
211bosxcls	3	7.0	157.0	90.0	13.5	42.5	33.5	31.0	17.5	15.5	40	298	16.5	225	8	212	7.5	4.0	2.0	1.0
211bosxcls	4	7.0	154.0	85.5	13.0	40.0	35.5	22.5	14.5	10.5	34	338	15.5	194	7	195	6.0	3.5	2.0	1.0

Farm ID	Layer	Sc	V	Cr	Co	Ni	Cu	Zn	As	Br	Rb	Sr	Y	Zr	Nb	Ba	Pb	Th	U	Mo
										(mg kg^{-1})										
284petkrfs	1	53.5	194.0	262.5	27.5	88.0	83.5	141.0	14.5	1.5	59	70	29.0	176	9	340	16.5	7.5	2.0	2.0
284petkrfs	2	41.0	162.0	229.0	25.5	76.0	62.0	84.5	13.0	1.0	50	78	24.0	218	8	306	13.0	5.5	2.0	2.0
284petkrfs	3	33.0	140.5	214.5	25.5	73.0	52.5	67.0	11.5	1.0	47	63	22.0	267	8	296	11.5	4.0	2.0	1.0
284petkrfs	4	23.0	134.0	226.5	23.0	74.0	46.5	43.5	12.0	2.0	43	60	20.0	300	8	277	10.5	5.0	2.0	2.0
140troklkn	1	17.5	90.0	74.5	18.0	41.5	29.5	111.5	18.0	2.5	108	218	32.5	255	12	550	25.5	12.5	3.5	2.0
140troklkn	2	9.5	84.0	73.0	17.5	37.5	26.0	107.5	17.0	2.0	110	316	31.5	240	12	564	25.5	12.0	5.0	2.0
140troklkn	3	14.0	84.5	55.5	16.5	33.0	25.5	118.0	22.0	1.0	130	279	31.0	213	13	582	24.5	14.0	4.5	1.0
140troklkn	4	11.5	80.5	51.5	15.0	31.5	24.0	122.0	18.0	1.0	128	316	31.0	217	13	582	26.0	14.0	4.5	2.0
131smibssv	1	9.5	58.5	74.5	15.5	35.5	27.5	76.5	13.0	5.5	78	193	27.5	342	10	486	17.5	8.0	2.0	1.0
131smibssv	3	9.0	67.5	78.0	15.5	40.0	30.5	77.0	15.5	9.0	81	202	30.0	325	11	484	18.0	9.5	2.0	2.0
131smibssv	4	20.0	80.0	91.0	22.0	48.0	36.5	99.0	14.0	10.5	89	182	33.5	288	12	511	20.5	10.0	2.0	2.0
131smibssv	5	10.0	111.5	97.0	19.0	48.5	39.5	108.0	16.5	2.5	94	431	30.5	214	10	517	17.0	9.5	4.5	1.0
131smibssv	1	9.0	59.5	89.0	14.5	42.5	29.5	98.5	13.5	18.0	86	303	28.5	289	11	507	20.0	8.0	2.5	2.0
131smibssv	2	7.0	90.5	93.0	15.0	42.5	24.5	72.5	19.5	1.0	94	392	26.0	264	10	488	18.5	9.0	4.0	3.0
131smibssv	3	11.0	93.0	95.0	14.5	44.0	24.0	74.0	19.0	1.5	95	375	26.0	270	10	486	17.5	9.0	3.0	4.0

Appendix H: Detailed mineralogical (XRD) data of the clay fraction

Farm ID	Layer	Quartz	Mica	Plagio-clase	K-feldspar /rutile	Calcite	Apo-phyllite	Anatase	Ankerite	Halite	Dolomite	Kaolinite	Smectite	Pyroxene	Gypsum	Anda-lusite	Illite/smec-tite / inter-stratification	Goethite	Serpentine	Clinochlore
											(%)									
p013bradlpn	1	28.9	33.0	18.9	19.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p001brawtkr	1	37.4	24.4	19.2	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p002bulwltv	1	27.3	31.1	19.4	0.0	9.7	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p004bullmrl	1	17.6	16.2	12.4	13.1	5.3	0.0	0.0	0.0	11.7	7.6	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0	0.0
p009deaqwgg	1	34.6	35.4	24.4	0.0	0.0	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p009deaqwgg	1	23.4	23.7	17.2	16.8	7.0	0.0	0.0	11.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p011petmrtn	1	24.1	23.8	14.8	0.0	5.2	0.0	0.0	0.0	7.7	8.3	16.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p010jacrtrv	1	15.3	19.4	14.4	14.3	5.2	0.0	0.0	0.0	12.8	9.8	0.0	0.0	0.0	0.0	8.8	0.0	0.0	0.0	0.0
p008oppdmsh	1	20.5	0.0	17.2	0.0	16.6	0.0	0.0	10.2	10.1	0.0	0.0	25.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p007lucwtrp	1	24.1	23.8	14.8	0.0	5.2	0.0	0.0	0.0	7.7	8.3	16.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p005petbrkp	1	41.2	34.1	24.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p012redbgnv	1	30.3	29.4	19.6	20.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p014blo7dms	1	24.1	25.3	16.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2	0.0	16.2	0.0	0.0	0.0	0.0	0.0	0.0
p003bulmgkr	1	20.6	0.0	18.7	16.5	5.8	0.0	0.0	0.0	9.9	0.0	0.0	28.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p006bftddmm	1	29.4	24.2	15.7	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.6	0.0	0.0	0.0	0.0	0.0	0.0
p006bftddmm	1	36.3	34.2	0.0	12.8	6.7	0.0	0.0	0.0	10.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	28.6	28.8	17.0	17.3	0.0	0.0	0.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	24.6	28.8	19.7	0.0	14.2	0.0	0.0	0.0	0.0	12.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	23.1	31.8	16.3	0.0	8.6	0.0	3.9	16.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	15.8	21.6	14.4	14.5	12.9	0.0	0.0	0.0	11.4	9.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	30.9	25.3	14.5	0.0	0.0	0.0	0.0	13.9	0.0	0.0	15.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
p015kimgrsp	1	23.2	25.3	13.8	12.1	0.0	0.0	0.0	9.8	0.0	0.0	15.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
321hoppplr	1	16.0	21.0	10.0	0.0	9.0	0.0	0.0	0.0	0.0	7.5	13.0	0.0	0.0	0.0	12.0	23.0	0.0	14.0	0.0
321hoppplr	2	16.5	18.0	9.0	0.0	10.5	0.0	0.0	0.0	0.0	7.0	11.0	0.0	14.0	0.0	0.0	19.5	0.0	0.0	13.0
321hoppplr	3	16.5	19.5	10.0	0.0	10.0	0.0	0.0	0.0	0.0	7.0	14.0	0.0	0.0	0.0	12.0	21.5	0.0	0.0	18.0
321hoppplr	4	20.5	22.5	13.5	0.0	10.0	0.0	0.0	0.0	0.0	7.0	13.0	0.0	0.0	0.0	0.0	24.0	0.0	0.0	0.0
222phibthl	1	29.5	29.0	20.5	0.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.0	0.0	0.0	0.0
222phibthl	2	22.5	23.5	15.5	0.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.5	0.0	0.0	0.0
222phibthl	3	21.0	25.0	15.0	0.0	13.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
222phibthl	4	21.5	31.5	14.0	0.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
259edemdws	1	30.0	26.0	16.5	0.0	0.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	5.0	0.0	0.0
259edemdws	2	20.5	26.0	15.5	0.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.0	6.0	0.0	0.0
259edemdws	3	25.5	31.0	15.0	0.0	20.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.0	5.0	0.0	0.0
259edemdws	4	29.0	24.5	15.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.0	5.0	0.0	0.0
211bosxcls	1	23.5	29.0	17.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.0	0.0	0.0	0.0	32.0	0.0	0.0	0.0
211bosxcls	2	21.5	26.5	16.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.0	0.0	0.0	0.0
211bosxcls	3	16.0	26.5	12.5	0.0	6.0	0.0	0.0	0.0	0.0	12.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0
211bosxcls	4	26.0	45.0	0.0	0.0	13.0	0.0	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Farm ID	Layer	Quartz	Mica	Plagio-clase	K-feldspar /rutile	Calcite	Apo-phyllite	Anatase	Ankerite	Halite	Dolomite	Kaolinite	Smectite	Pyroxene	Gypsum	Anda-lusite	Illite/smec-tite / inter-stratification	Goethite	Serpentine	Clinochlore
											(%)									
284petkrfs	1	30.5	28.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.0	32.5	0.0	30.0	0.0
284petkrfs	2	23.0	26.5	17.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.0	0.0	21.0	0.0
284petkrfs	3	26.0	34.0	21.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.0	0.0	23.0	0.0
284petkrfs	4	19.0	21.5	14.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.5	6.0	17.5	0.0
140troklkn	1	25.5	28.0	15.0	0.0	5.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	26.5	6.0	0.0	0.0
140troklkn	2	23.5	27.5	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.5	6.0	0.0	0.0
140troklkn	3	20.5	24.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.5	4.5	15.0	0.0
140troklkn	4	19.5	24.5	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.5	6.0	15.0	0.0
131smibssv	1	20.0	25.0	14.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	30.5	0.0	0.0	0.0	21.0	0.0	0.0	0.0
131smibssv	3	21.5	24.0	14.5	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0	23.5	0.0	0.0	0.0
131smibssv	4	25.5	27.5	15.5	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.5	0.0	0.0	0.0
131smibssv	5	22.0	26.5	15.0	0.0	5.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	27.5	0.0	0.0	0.0
131smibssv	1	25.0	24.0	16.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.5	0.0	16.0	0.0
131smibssv	2	26.0	26.5	17.5	0.0	0.0	0.0	0.0	0.0	7.0	0.0	0.0	32.0	0.0	0.0	0.0	28.0	0.0	0.0	0.0
131smibssv	3	24.5	27.0	16.5	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	31.0	0.0	0.0	18.0	27.0	0.0	0.0	0.0

Appendix I: Detailed microbiological data

Farm ID	Layers	FDA	FDA(2017)	Active C
p013bradlpn	1	63.0	58.8	637.8
p001brawtkr	1	14.2	11.0	592.8
p002bulwltv	1	83.9	85.2	519.1
p004bullmrl	1	34.3	118.2	600.9
p009deaqwgg	1	140.0	15.6	288.1
p009deaqwgg	1	141.4	56.4	128.3
p011petmrtn	1	66.1	36.2	332.6
p010jacrtrv	1	71.2	17.4	549.2
p008oppdmsh	1	37.4	68.8	485.6
p007lucwtrp	1	24.2	135.5	685.0
p005petbrkp	1	342.3	92.6	482.2
p012redbgnv	1	51.7	135.4	486.3
p014blo7dms	1	85.2		370.7
p014blo7dms	1		92.8	
p003bulmgkr	1	89.7	97.9	168.9
p006bftddmm	1	128.6	54.1	189.1
p006bftddmm	1	55.6	69.7	562.9
p015kimgrsp	1	38.5	41.5	506.5
p015kimgrsp	1	57.9	15.6	474.3
p015kimgrsp	1	47.6	15.7	443.9
p015kimgrsp	1	42.3	27.0	343.3
p015kimgrsp	1	4.8	10.7	500.2
p015kimgrsp	1	20.6	5.4	551.7
321hoppplr	1		76.5	
222phibthl	1		21.6	
259edemdws	1		9.7	
211bosxcls	1		90.2	
284petkrfs	1		16.2	
140troklkn	1		48.9	
131smibssv	1		48.7	
131smibssv	1		0.2	