Effects of ash from native and alien plants on phytoplankton biomass and mosquito abundances: A mesocosm experiment

by

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ABSTRACT

Wildfires are natural or anthropogenic phenomena increasing at alarming rates globally due to land–use alterations, droughts, climatic warming, hunting and biological invasions. Whereas wildfire effects on terrestrial ecosystems are marked and relatively well–studied, ash depositions into aquatic ecosystems have often remained overlooked, but have the potential to significantly impact bottom–up processes and effects on semi–aquatic taxa such as mosquitoes. This study assessed (i) ash–water– phytoplankton biomass dynamics and (ii) post–colonization mosquito abundances using six plant species [i.e., three natives (apple leaf *Philenoptera violacea*, Transvaal milk plum *Englerophytum magalismontanum*, quinine tree *Rauvolfia caffra*) and three aliens (lantana *Lantana camara*, gum *Eucalyptus camaldulensis*, guava *Psidium guajava*)] based on a six–week mesocosm experiment with different ash concentrations (1 and 2 g L^{-1}). We assessed concentrations of chemical elements, i.e., N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B from ash collected, and observed significant differences amongst the species. High concentrations of P, K, Mn, Fe, Cu, Zn and B were recorded from Transvaal milk plum ash and low concentrations of P, K, Ca, Mg, Cu and Zn were recorded from apple leaf. An increase in phytoplankton biomass (using chlorophyll–a [chl-a] concentration as a proxy) for all treatments i.e., 1 and 2 g L^{-1} for all plant species ash was observed a week after, followed by decreases in the following weeks, with the exception of 2 g L^{-1} for lantana, gum and control. Silicate concentrations (i.e., used as a proxy for diatom abundance) showed increasing patterns among all ash treatments, with exception of controls. However, no clear patterns were observed between native and alien plant ash on both chlorophyll-a (chl–a) and silicate concentrations. We found that ash has notable effects on water

chemistry, particularly nitrate, which increased throughout the weeks, whereas, pH and conductivity were high at lower ash concentrations. The impacts of ash on water chemistry, chl–*a* and silicate concentrations varied with individual species and the amount of ash deposited into the system. Overall, there was no statistically clear difference in colonization between ash from native and alien species. We recorded colonization by two mosquito genera (i.e., *Culex* spp., *Anopheles* spp.), with *Culex* generally much more abundant than *Anopheles*. Few differences were identified among the plants, with statistically clear effects of ash type and concentration on larval and pupal stages. High *Culex* egg and larval abundances were shown in lantana and apple leaf treatments compared to controls, and milkplum versus controls for pupae of both genera. Further research is required to elucidate the influence of nutrient inputs from different ash species on vector mosquito population and phytoplankton dynamics.

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ACKNOWLEDGEMENTS

DECLARATION

I <u>TAKALANI VINCENT NETSHITUNI</u> hereby declares that the dissertation for Master of Environmental science at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this university or any other university, that it is my own work and all reference material contained therein has been duly acknowledged.

Signature: _____   Date: <u>24/11/2022</u>

PREFACE

This thesis comprises a general introduction (Chapter 1), results (Chapter 2–3) and a general synthesis (Chapter 4). The combined reference list at the end of the thesis ensures limited repetition. The result sections were organised as scientific papers, which are currently *published* (see below):

Publication list

1. **Takalani V Netshituni**, Ross N Cuthbert, Farai Dondofema, and Tatenda Dalu. 2022. Effects of wildfire ash from native and alien plants on phytoplankton biomass. *Science of the Total Environment*. **Published**.

2. **Takalani V Netshituni**, Ross N Cuthbert, Farai Dondofema, and Tatenda Dalu. 2022. Assessing the effects of native and alien plant ash on mosquito abundance. *Ecology and Evolution*. **Published**.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Wildfires are natural phenomena occurring in most biomes each year (Smith et al., 2011), originating from both natural i.e., lightning and anthropogenic activities i.e., post–harvest burning of agricultural fields residues and hunting (Pinto et al., 2004; Dalu et al. 2017). Essentially, wildfires play a vital role in structuring of biomes (Pinto et al., 2004; Juli et al., 2008), but also pose a threat to biodiversity and infrastructure (Smith et al., 2011). The frequency of these fires is facilitated by a combination of various ecosystem factors, for instance, temperature, vegetation density or fuel load, soil moisture, wind speed and general climatic conditions (Running, 2006; Wehner et al., 2017). One of the role players in the alteration of wildfire frequency is alien invasive plants by continuously providing more fuel which in most cases is easy to ignite (Tunison, D'Antonio and Loh, 2000; Brooks et al., 2004; Bell, Ditomaso and Brooks, 2009).

During wildfire, some components (organic and inorganic) are released into the air while some residuals remain on the ground and are ultimately transported by post–fire activities of wind and water (Silva et al., 2015). The soluble components and particulate matter leached from ash together with its underlying soil washed into the aquatic ecosystems may alter water chemistry (Smith et al., 2011; Balfour et al.,, 2014; Brito et al., 2017; Harper et al., 2019).

Aquatic ecosystem is a broad term that encompasses freshwater ecosystems i.e., lakes, ponds, reservoirs, rivers, and wetlands, but it also refers to coastal and marine

ecosystems i.e., ocean, estuaries, salt marshes, coral reefs, and mangroves (Irfan and Alatawi, 2019). The biodiversity in aquatic ecosystems ranges from plankton, which are free–floating organisms, nektons (strong swimmers) and benthos (bottom dwellers) (Kumar et al., 1989; Miller and Spoolman, 2012). Such biodiversity is largely influenced by aquatic environmental factors such as dissolved oxygen, sunlight availability, salinity, pH, temperature, and nutrients (Solomon et al., 2015).

There is a strong link between the terrestrial and aquatic ecosystems, which entails a complex exchange of materials i.e., through surface runoff (Wetzel, 2001). The interaction between terrestrial and aquatic ecosystem often has an impact on water chemistry. Aquatic ecosystems are mostly affected by contaminants of various origins (Vidal et al., 2019), with wildfires being one of the major role players in changing water quality thereby affecting total functionality of the aquatic ecosystems (Minshall et al., 1989; Pinto et al.,2009; Bixby et al., 2015). The changes in the water chemistry due to wildfire ash affect the total functionality of the aquatic ecosystems. There are several methods to monitor the status of these ecosystems such as the use of macroinvertebrates, algae (primary productivity), and fish (Lindell and Welch, 1992; Minshall, 2003; Gresens et al., 2010).

Different groups of aquatic and semi–aquatic macroinvertebrates react differently to the disturbance depending on the type and magnitude of disturbance in the ecosystem (Javier, 2006). However, the most commonly used group are the benthic macroinvertebrates (Sharma and Rawat, 2009). The rate of colonization by the macroinvertebrates may somehow indicate the quality of the water or the health status of an ecosystem (Wallace et al., 1996).

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Change in water chemistry has notable impacts on autotrophs. Primary productivity in aquatic ecosystems is influenced by various factors such as light, temperature, nutrients availability, physical transport processes and herbivory by zooplankton (Lange et al., 1983) which are likely to be affected by ash deposition.

1.2   Problem statement

The effects of wildfires on the aquatic ecosystem have not been intensively studied (Pinto et al., 2004; Quintana, 2016) especially post–fire chlorophyll–a (Chl-*a*) biomass and mosquito abundances. However, attention to wildfires as a source of contaminants to aquatic ecosystems has increased drastically as a result of their production of harmful pyrolytic substances and the ultimate fate of such substances (Silva et al., 2015). In most regions, wildfires are one of the major ash producers which is ultimately leached into various aquatic environments thereby posing either negative or positive impacts (Urbanski et al., 2008; Brito et al., 2017). The current study focuses on the effects of ash produced from native and alien plants on the phytoplankton biomass and the mosquito abundances after colonization.

1.3   Aim and objectives

1.3.1 Research aim

The main aim of the current study is to investigate the effects of ash produced from native and alien plant species on the water chemistry, phytoplankton biomass and evaluating post–colonization mosquito abundances in lentic aquatic ecosystems.

1.3.2 Specific objectives

- To assess the effects of plant ash on the water chemistry.

- To evaluate the ash chemical composition produced from different plants

- To investigate the changes in phytoplankton biomass following ash (native and alien) deposition into the water.

- To assess the effects of native and alien plant ash on post–colonization mosquito abundances

1.4 Research hypotheses

- The amount of wildfire ash deposited into the water decreases the water quality and increases primary productivity by the lentic aquatic ecosystem.

- Ash will positively influence the abundance of mosquitoes owing to the improved habitat suitability which may attract adult mosquitoes for oviposition and benefit their development. Moreover, invasive alien plants will further promote greater abundances of vector mosquitoes than the native plant species, given the provisioning of higher biomass.

1.5 Thesis outline

As highlighted in the preface, the thesis consists of general introduction (Chapter 1), two data chapters (Chapters 2 and 3) and a general conclusions chapter (Chapter 4). Chapters 2 and 3 of the thesis focus on the *Effects of wildfire ash from native and alien plants on phytoplankton biomass* and *Assessing the effects of native and alien plant ash on mosquito abundance*, respectively. Finally, Chapter 4 provides an overall synthesis of the thesis and provides key conclusions and recommendations.

CHAPTER 2: EFFECTS OF WILDFIRE ASH FROM NATIVE AND ALIEN PLANTS ON PHYTOPLANKTON BIOMASS

2.1 Introduction

For the past million years hominids have been the major igniters of wildfires (accidentally or for management purposes), with other natural processes such as lightning igniting approximately 10% of the wildfires in the savannas (Cassidy et al., 2022). These phenomena are increasing at alarming rates globally, caused by vegetation changes with alien invasive plants contributing more by continuously modifying fuel load, fire continuity and ignitability of vegetation communities, human population density, agricultural pre-cultivation and post-harvest burning ,uncontrolled fires emerging from hunting activities and climatic conditions such as change in rainfall seasonality and extreme droughts (Pinto et al., 2004; Archibald et al., 2009; Smith et al., 2011; Dalu et al., 2017). Wildfires are essential for biome structuring (Pinto et al., 2004; Juli et al., 2008); however, they can pose severe threats to biodiversity and habitat quality (Smith et al., 2011; Nunes et al., 2018; Oliveira–Filho et al., 2018; Carvalho et al., 2019; Rhoades et al., 2019). In the process of burning, wildfires release large quantities of organic and inorganic compounds into the natural environment (Ugurlu, 2004; Ferrer et al., 2021; Pelletier et al., 2022). Wildfire ash constitutes the remaining particulate residue, usually deposited on the ground from burnt wildland biomass, and consists of mineral ions and charred organic matter (Bodí et al., 2014). The ash chemical composition mainly relates to the vegetation type and parts burnt (e.g., leaves, barks and roots) (Smith et al., 2011; Hohner et al., 2019). The principal compounds of ash are calcium, phosphates, carbonates, silicates, oxides, sulphates, and amorphous phases that either exist as primary minerals in the

plant materials or transform because of the heating during a fire (Demeyer et al., 2001; Balfour, 2013). Thus, the severity of burning will determine the ash organic carbon content, for example, at low combustion completeness; the ash is organic carbon–rich, whereas at high combustion completeness, most organic carbon is volatilised (Bodí et al., 2014).

Whereas much of these compounds are being deposited directly into terrestrial environments, wildfire ash is also dispensed into aquatic ecosystems (Rulli and Rosso, 2007; Bodí et al., 2014; Hahn et al., 2019). The ash deposited on the ground after wildfires can easily be transported to various ecosystem types via post–fire vectors, such as water or wind (Silva et al., 2015). Post–fire rainfalls and subsequent runoff act as a primary transport mechanism for ash into aquatic ecosystems (Wetzel, 2001). Thus, the soluble compounds and particulate matter leached from ash enter aquatic environments, affecting water chemistry variables such as nutrients, metals, and ions (Smith et al., 2011; Bodí et al., 2014; Brito et al., 2017; Harper et al., 2019). The insoluble ash particles from wildfires are also responsible for dissolved oxygen depletion in aquatic ecosystems, as they absorb sunlight and consequently increase water temperatures (Flynn et al., 2018). Ash has been highlighted to change water quality, thereby affecting aquatic ecosystems' function and structure (Nunes et al., 2018; Rhoades et al., 2019). The ecological functions and structuring of aquatic ecosystems are supported fundamentally by phytoplankton.

In aquatic ecosystems, phytoplankton has the greatest contribution towards total primary production, forming the basic support for aquatic food webs (Ezekiel et al., 2011). Numerous studies (e.g., Silva et al., 2015; Kramer et al., 2020; Vidal et al.,

2021) have reported both beneficial and harmful effects of wildfire ash on the phytoplankton community composition and biomass due to its chemical composition. Among ash properties, nitrate and phosphate are widely reported to facilitate aquatic plant growth and pose a severe risk of eutrophication within aquatic environments, thus bolstering primary productivity (Pinto et al., 2004; Smith et al., 2011; Vajda et al., 2020). Aquatic primary productivity, mostly by phytoplankton, in turn, depends on abiotic and biotic factors such as carbon dioxide, pH, temperature, nutrients, solar radiance, and herbivory (Häder et al., 2014; Dalu et al., 2022), and these factors can be substantially changed following the introduction of ash into the aquatic ecosystems.

Wildfires and invasive alien plants have been highlighted to interact, producing marked impacts on terrestrial and riparian environments, and leading to changes in how landscapes are managed (D'Antonio and Vitousek, 1992; Brooks and Matchett, 2006; Brunson and Tanaka, 2011; Weltz et al., 2011). Several invasive alien plant species are known to alter the extent, frequency, intensity, type, and/or seasonality of wildfires, resulting in either increased or decreased fire prevalence across the landscape (Tunison et al., 2000; Brooks et al., 2004; Brooks and Matchett, 2006; Bell et al., 2009). For example, whereas wildfires in native riparian vegetation tend to occur only during extreme drought periods and typically remain in the surface vegetation, whereas wildfires within riparian zones dominated by invasive alien plants can occur over a broader climatic and environmental range, often spreading into riparian woodlands and forest canopies at high intensities (Bell et al., 2009). In turn, their effects may cause species composition shifts, which can have ecosystem–wide effects (Brooks and Matchett, 2006; Faccenda and Daehler, 2021).

Globally, wildfires affect approximately 350 million hectares annually, with Africa accounting for about half of this area burnt (Attri et al., 2020). In South Africa alone, about 1.18% of the national vegetated land surface is burnt annually, and the frequency of fires varies with ecosystem type. For example, wildfires can range from every year in little–grazed, moist grassland ecosystems, 10‑20 years in the fynbos, and rarely in desert environments (Forsyth et al., 2010). South Africa records an average of between 35 000 and 40 000 fires per year, but the number could be much higher due to unreported events from human–induced activities (Strydom and Savage, 2016). Compounding this problem, South Africa is continuously experiencing new annual plant invasions at alarming rates, with suggestions that some of these invasive alien plant species may further modify fire regimes (Forsyth et al., 2010). This combination of factors makes South Africa an ideal, practical case study for assessing and comparing how native and invasive alien plants can affect aquatic ecosystem functioning from ash inputs.

The present study thus aimed to assess ash–water–chlorophyll–a dynamics using six plant species, of which three were native (apple leaf *Philenoptera violacea*, Transvaal milk plum *Englerophytum magalismontanum*, quinine tree *Rauvolfia caffra*) and three were invasive alien plants (lantana *Lantana camara*, red river gum *Eucalyptus camaldulensis,* guava *Psidium guajava*), each frequently known to occur near or around aquatic ecosystems. Hence, the ash from these plants is most likely to be deposited into aquatic ecosystems during and post–fire events. The study aimed to assess the effects of native and alien leaf ash of different concentrations on phytoplankton biomass using chlorophyll–*a* concentrations as a proxy, as well as silicate, which can be used as an indicator for diatom abundance. We thus assessed

(i) ash chemical properties from different plant species, (ii) effects of ash concentrations on water physicochemical parameters, and (iii) chlorophyll–*a* and silicate dynamics as proxies of phytoplankton and diatom concentrations. We hypothesized that (i) ash will lead to increased localized phytoplankton biomass, with decreased water quality within a few days of ash addition due to increased nutrient, ion, and oxide contents. Further, we expected (ii) ash produced from alien plant species to facilitate phytoplankton and diatom concentration increases more than natives, due to faster plant growth rates which will lead to high nutrient, ion and oxide uptake and accumulation.

2.2 Materials and methods

2.2.1 *Experimental design*

The experiment was conducted at the University of Venda Department of Geography and Environmental Sciences Atrium (−22.977550, 30.443851) using 64 × 12 L buckets (⌀ 25 cm and 30 cm depth). The buckets were filled with 10 L filtered (63 µm mesh to remove zooplankton) river water collected from the Mvudi River (−22.983544, 30.443331). Five grams of slow–release Wonder plant booster all–purpose 3:2:1 (N:P:K ratio) fertiliser (Wonder Garden Care, Kempton Park) were added into the water to facilitate 'baseline' phytoplankton growth over 30 days before the start of the experiment. Leaves and twigs (thereafter referred to as leaves) were collected from three native (i.e., *R. caffra, E. magalismontanum, P. violacea*) and three alien (i.e., *L. camara, P. guajava, E. camaldulensis*) plant species before being sundried for 40 days in an open yard at Thohoyandou Unit C (September to October 2020). Once the leaves had dried, each plant species was separately placed inside a metal bucket, then ignited with a matchstick and allowed to burn for 50‑60 min to produce ash; the

fire intensity was not standardised across the species, but adequate in each case to produce a representative ash sample for experimentation. The fire was extinguished by covering each metal bucket with a lid. All the ash was collected separately per species after it had cooled down and placed into labelled ziplock bags to form the six individual ash treatments, and an additional seventh treatment (mixed) was made using equal proportions of the other six individual ash treatments. The seven different ash treatments were sent to a South African National Accreditation System (SANAS) certified laboratory i.e., BEMLAB to assess for the ash nutrient levels [nitrogen (N; %), phosphorus (P; %), potassium (K; %)] and metal contents [i.e., calcium (Ca; %), magnesium (Mg; %), sodium (Na; mg kg^{-1}), manganese (Mn; mg kg^{-1}), iron (Fe; mg kg^{-1}), copper (Cu; mg kg^{-1}), zinc (Zn; mg kg^{-1}), boron (B; mg kg^{-1})] (see Dalu et al., 2020a, 2020b for detailed methods).

The experiment used a randomised design, with eight species treatments [i.e., 3 native, 3 alien, 1 mixed, 1 control (no ash)] × 4 replicates ×2 ash concentrations (i.e., 1 and 2 g L^{-1}) and was run from 05 November 2020 to 10 December 2020. The ash concentrations were based on conservative estimates from Brito et al. (2017, 2021). At the end of the 30 days of phytoplankton growth, before adding ash, physicochemical variables were measured, and 100 mL water for chlorophyll–a determination was collected.

Every week during the experiment, a portable handheld multiparameter Cyberscan Series meter (Eutech Instruments, Singapore) was used to measure water conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH, temperature (°C), sodium chloride (ppm), oxidation–reduction potential (mV) and resistivity (Ω). After collecting

the first water samples (i.e., week 1 was ash–free), ash at 10 g or 20 g mass was randomly introduced into the individual buckets, except controls, where no ash was introduced.

We applied 10 g (1 g L^{-1}) and 20 g (2 g L^{-1}) to resemble real world scenarios, particularly in smaller water bodies where such concentrations are likely to be achieved, mostly after the first surface run offs following wildfires. However, we acknowledge that ash concentrations are likely hugely variable in waters empirically, owing to various hydrological processes, and the applied concentrations here are generally high and consequently represent 'worst case' scenarios. The choice of ash mass was to assess the different responses by chl–a and silicate concentrations (as proxies for phytoplankton productivity) to varying ash concentrations. To compensate for water loss, borehole water was used to top up the buckets to initial levels, by replacing the water taken due to sampling or to normal evaporation processes. These water additions were well–balanced among treatments.

2.2.2 *Nutrient and silicate analyses*

Approximately 50 mL of water samples was collected from each treatment and replicated weekly for nutrient (ammonium, nitrate, phosphates) and silicate analyses. The nutrients and silicate were analysed at NRF SAEON Elwandle Node Coastal Biogeochemistry Laboratory (Port Elizabeth, now renamed Gqeberha) using an Auto–Analyser model AA3 segmented flow colourimetry (SEAL Analytical). Phosphates were analysed using the calorimetric method, with readings taken at 880 nm, at a test range of 0–50 µg L^{-1}. Nitrate was analysed, reducing nitrate to nitrite using a

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copper-cadmium redactor column (Armstrong et al., 1967) and with a test range of 0‒50 µmol L^{-1}. Ammonium concentration was based on the Berthelot reaction, with measurements taken at 660 nm (test range 0‒10 µmol L^{-1}). Finally, silicate concentrations were analysed based on reducing silico–molybdate in an acidic solution to molybdenum blue by ascorbic acid according to Grasshoff et al. (1983), at a test range of 0‒41 µmol L^{-1}.

2.2.3 *Chlorophyll–a concentration determination*

Chlorophyll–a concentration was determined weekly as a proxy for phytoplankton biomass from all treatment buckets. The 100 mL water sample from each mesocosm was filtered (vacuum <5 cm Hg) through 0.7 µm pore size (diameter 47 mm) reinforced glass fibre filters (GF/F; GIC Scientific, Roodepoort). After filtration, the GF/F filters were inserted in 15 mL tubes containing 10 mL of 90% acetone solution and then stored in a freezer for at least 24 h to allow for chl–*a* extraction. After 24 h, samples were centrifuged at 3000 rpm for 10 min before 2 mL was extracted from each sample to measure absorbance at wavelengths of 665 nm and 750 nm using SPECTROstar NANO (BMG LabTech GmbH, Ortenberg). Absorbance was measured through a 10 mm cuvette before and after acidification with 0.01 M hydrochloric acid (HCl). Absorbance readings were recorded before chl–*a* concentration and calculated based on Lorenzen (1967).

2.2.4 *Data analysis*

All data were assessed for normality and homogeneity of variance and were found to conform to parametric assumptions using the Shapiro‒Wilks W and Levene's tests. A

one–way ANOVA was used to analyze differences among treatments for the various nutrient and metal concentrations before the experiment. We tested whether there were significant experimental differences in physicochemical variables and, particularly, chl–*a* concentration and silicate among the study weeks (i.e., 1‑6), treatments (i.e., six species) and ash concentrations (i.e., 1 and 2 g L^{-1}) using factorial repeated measures ANOVA in STATISTICA version 8 (StatSoft Inc, 2007). Variables that were retained as significant were further tested, using Tukey's post–hoc analysis to assess differences among treatments and weeks. To evaluate relationships in chlorophyll–a and silicate concentrations among study treatments and ash weights, a Pearson correlation was carried out in SPSS v16 (SPSS Inc., 2007).

2.3 Results

2.3.1 *Leaf metal and nutrient concentrations*

In general, P, K, Mn, Zn, Cu and B concentrations were high in the Transvaal milk plum, with lantana and apple leaf having high N and Fe concentrations, respectively. Gum had high Ca, Mg and Na concentrations (Table 2.1). Low concentrations were generally observed in the gum (N, Fe), quinine tree (Na, Mn, B) and apple leaf (P, K, Ca, Mg, Cu, Zn) groups (Table 2.1). Using a one–way ANOVA analysis, significant differences ($p < 0.001$) were observed for all nutrient and metal plant concentrations across treatments, except P, which was not significant (F = 1.00, $p = 0.480$).

Table 2.1. Plant ash nutrient and metal concentrations (± standard deviation) before the start of the experiment, i.e., addition into the experimental mesocosms. Abbreviations: B – boron, Ca – calcium, Cu – copper, Fe – iron, K – potassium, Mg – magnesium, Mn – manganese, N – nitrogen, Na – sodium, P – phosphorus, Zn – zinc.

Parameter	SI unit	Lantana	Quinine tree	Guava	Gum	Transvaal milk plum	Apple leaf
N	%	1.01±0.02	0.72±0.01	0.41±0.01	0.24±0.01	0.28±0.01	0.93±0.04
P	%	0.84±0.01	1.19±0.02	0.62±0.02	0.82±0.01	0.92±0.01	0.22±0.01
K	%	4.34±0.51	5.68±0.11	4.23±0.02	5.72±0.01	7.25±0.01	1.47±0.04
Ca	%	11.30±0.42	7.71±0.01	15.48±0.04	21.10±0.14	10.15±0.07	6.05±0.04
Mg	%	1.53±0.08	1.21±0.01	2.08±0.1	3.42±0.01	2.30±0.02	1.02±0.02
Na	mg kg^{-1}	1255±63.6	604±1.4	1305±7.1	14630±56.6	13801±1.4	1150±42.4
Mn	mg kg^{-1}	1415±7.1	527.0±2.8	643.0±4.2	1905±7.8	6627±9.9	599.0±2.8
Fe	mg kg^{-1}	9053±17.7	9215±21.8	9705±7.1	6384±8.5	16587±4.9	22692±53.7
Cu	mg kg^{-1}	106.5±2.1	68.7±0.4	75.5±0.2	93.5±0.7	181.5±4.9	63.2±0.5
Zn	mg kg^{-1}	1116±19.8	1770±14.1	391.0±1.4	470.0±1.4	1334±50.9	288.5±2.1
B	mg kg^{-1}	221.0±1.4	137.0±3.5	323.5±3.5	720.5±3.5	767.0±2.8	318.0±2.8

2.3.2 *Water physicochemical variables variation*

Using repeated measures ANOVA, significant differences ($p < 0.05$) were observed among all physicochemical variables across the weeks, treatments and ash weights, with the exception for phosphate (week), temperature (treatment) and temperature (concentration), which were not significantly different ($p > 0.05$) as single terms (Table 2.2). No clear weekly patterns were observed for most physicochemical variables, with the exception of nitrate concentrations in both ash concentrations (Figures 2.1 and 2.2). Week 3 generally had high TDS, conductivity and temperature values recorded.

For 2 g L^{-1} concentration, the lantana and mixed groups had high phosphate concentrations (Figure 2.1). Ammonium, conductivity, and TDS concentrations were low for the gum and Transvaal milk plum, whereas the same ash treatments recorded high pH, resistivity and nitrate values (Figure 2.1). The pH and conductivity of 1 g L^{-1} ash treatment showed an increasing trend across weeks. The controls and apple trees had high phosphate concentrations, whereas slightly lower concentrations were observed for the Transvaal milk plum (Figure 2.2). Similarly, lantana had high nitrate concentrations at week 6, but with very high error margins probably due to a one replicate outlier. Control, mixed, and apple tree had high ammonium concentrations, whereas guava had high resistivity. The TDS, conductivity and temperature were high at week 3, whereas week 1 had low nitrate, ammonium, pH, TDS, and conductivity (Figure 2.2).
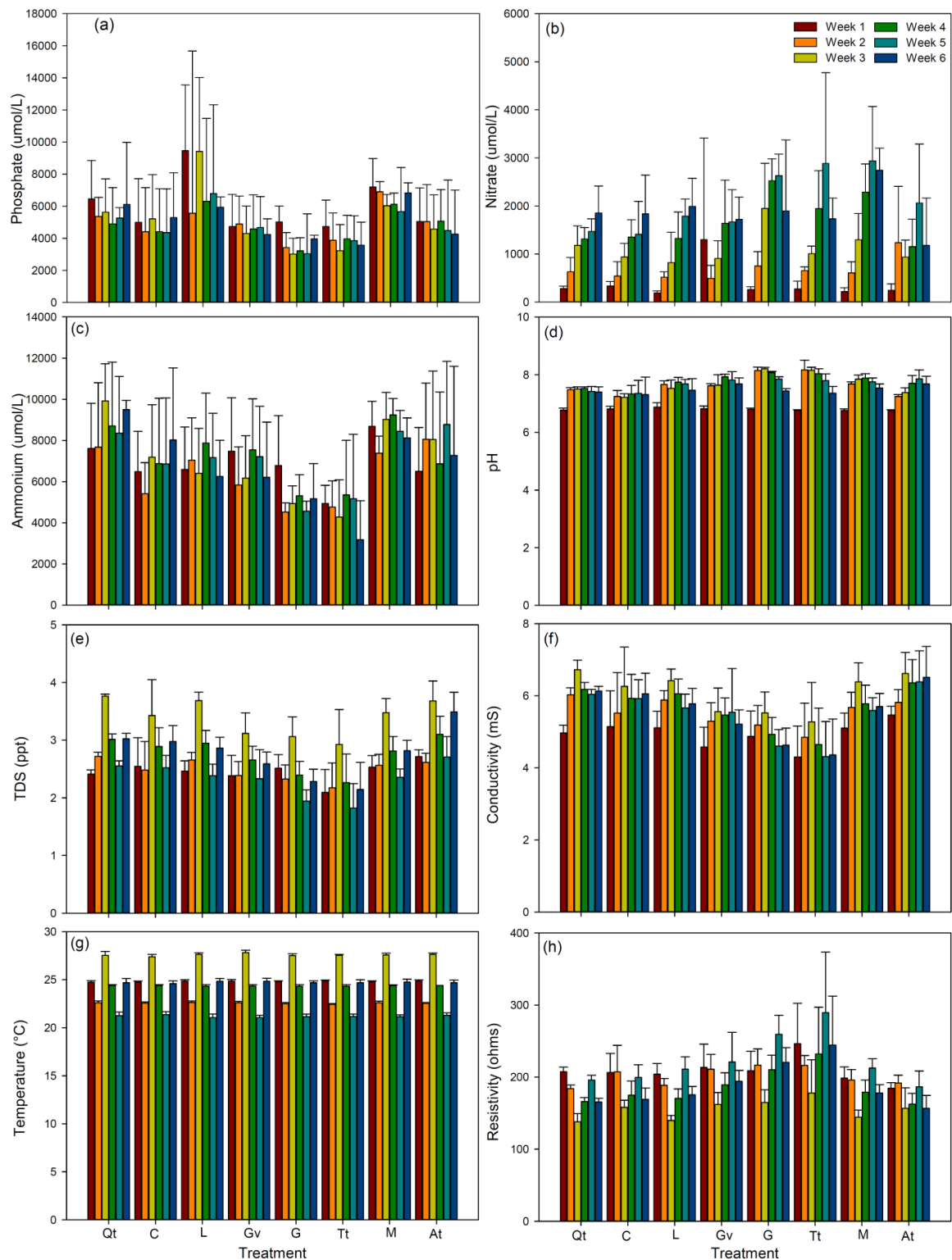
Figure 2.1. Physicochemical variables among the 2 g L^{-1} ash treatments over the study period. Abbreviations: Qt – quinine tree, C – control, L – lantana, Gv – guava, G – gum, Tt – Transvaal milk plum, M – mixed, At – Apple leaf
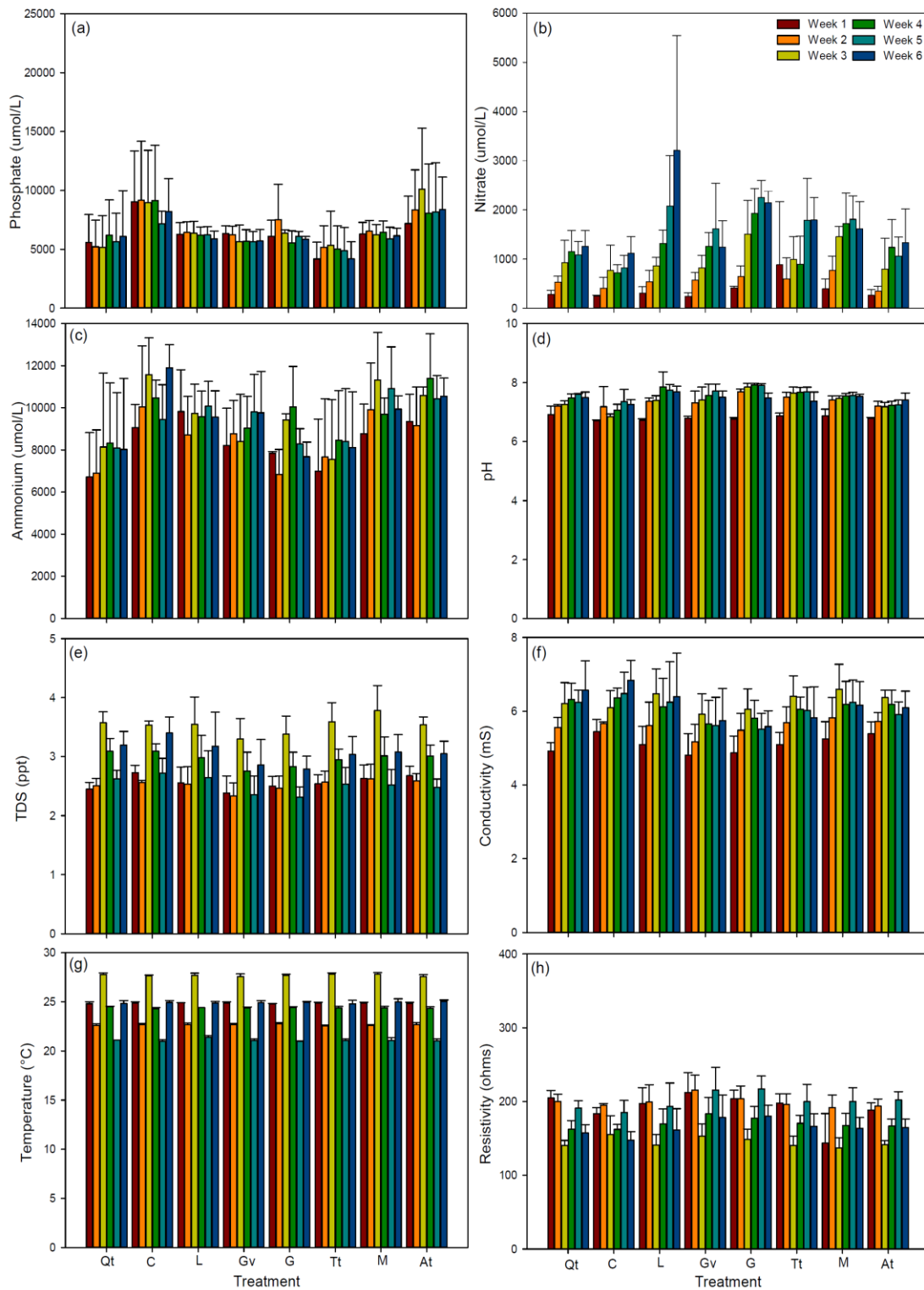
Figure 2.2. Physicochemical variables among the 1 g L^{-1} ash treatments over the study period. Abbreviations: Qt – quinine tree, C – control, L – lantana, Gv – guava, G – gum, Tt – Transvaal milk plum, M – mixed, At – Apple leaf

Table 2.2. Repeated measures ANOVA based on physicochemical variables for a week, treatment and ash. Bold values indicate significant differences at $p < 0.05$. Abbreviations: TDS – total dissolved solids, ORP – oxygen reduction potential

Variable	Week		Treatment		Ash		Week × Treatment		Week × Ash		Treatment × Ash		Week × Treatment × Ash	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Phosphate	0.52	0.757	**5.349**	**<0.001**	**4.380**	**0.037**	0.230	1.000	0.630	0.677	**8.612**	**<0.001**	0.258	1.000
Nitrate	**47.89**	**<0.001**	**6.322**	**<0.001**	**4.786**	**0.030**	1.384	0.083	0.835	0.526	**2.484**	**0.018**	0.914	0.612
Nitrite	**29.99**	**<0.001**	**6.354**	**<0.001**	**13.765**	**<0.001**	1.442	0.059	1.308	0.261	**4.643**	**<0.001**	0.966	0.528
Ammonium	**2.35**	**0.041**	**9.751**	**<0.001**	**37.452**	**<0.001**	0.419	0.999	0.572	0.721	**14.767**	**<0.001**	0.368	1.000
pH	**117.82**	**<0.001**	**23.574**	**<0.001**	**25.594**	**<0.001**	**3.097**	**<0.001**	**5.333**	**<0.001**	**6.237**	**<0.001**	1.291	0.137
TDS	**3.63**	**0.003**	**3.104**	**0.004**	**4.227**	**0.041**	**2.036**	**0.001**	**3.607**	**0.004**	**3.095**	**0.004**	**2.035**	**0.001**
Conductivity	**22.60**	**<0.001**	**15.795**	**<0.001**	**14.250**	**<0.001**	0.735	0.863	1.874	0.099	**7.694**	**<0.001**	0.472	0.995
ORP	**135.33**	**<0.001**	**29.496**	**<0.001**	**30.601**	**<0.001**	**3.648**	**<0.001**	**5.988**	**<0.001**	**6.414**	**<0.001**	**1.844**	**0.004**
Resistivity	**44.15**	**<0.001**	**14.025**	**<0.001**	**23.202**	**<0.001**	1.075	0.364	1.640	0.150	**9.604**	**<0.001**	0.742	0.855
Temperature	**6947.71**	**<0.001**	0.742	0.636	1.850	0.175	0.446	0.997	0.475	0.795	1.167	0.322	1.381	0.085
Salinity	**22.78**	**<0.001**	**15.230**	**<0.001**	**16.718**	**<0.001**	0.681	0.914	1.665	0.144	**9.473**	**<0.001**	0.458	0.997

2.3.3 *Chlorophyll–a and silicate dynamics*

Chlorophyll–*a* concentrations generally increased from week 1 to 2, with 1 g L^{-1} guava and apple leaf increasing to week 3 before decreasing for the following weeks (Figure 3a, b). However, the 2 g L^{-1} quinine tree, 2 g L^{-1} control, 2 g L^{-1} guava and 1 g L^{-1} mixed treatment showed different patterns (Figure 2.3a, b). Chlorophyll–*a* concentrations generally decreased in 2 g L^{-1} lantana and guava throughout the weeks.

Silicate concentrations generally increased over time, with very low concentrations recorded in the controls, although other treatments had slightly different patterns (Figure 2.3c, d). Among the native species, the quinine tree recorded relatively low silicate concentrations for both 2 g L^{-1} and 1 g L^{-1} ash weight, with the apple leaf and Transvaal milk plum showing high silicate concentrations. Guava generally recorded high silicate concentrations followed by lantana, with gum recording low silicate concentrations among the alien species.

Figure 2.3. Chlorophyll–*a* (a,b) and silicate (c,d) concentrations among the different treatments and ash concentrations [(a, c) – 2 g L^{-1}; (b, d) – 1 g L^{-1}] over a six–week experimental period. Abbreviations: Qt – quinine tree, C – control, L – lantana, Gv – guava, G – gum, Tt – Transvaal milk plum, M – mixed, At – Apple leaf
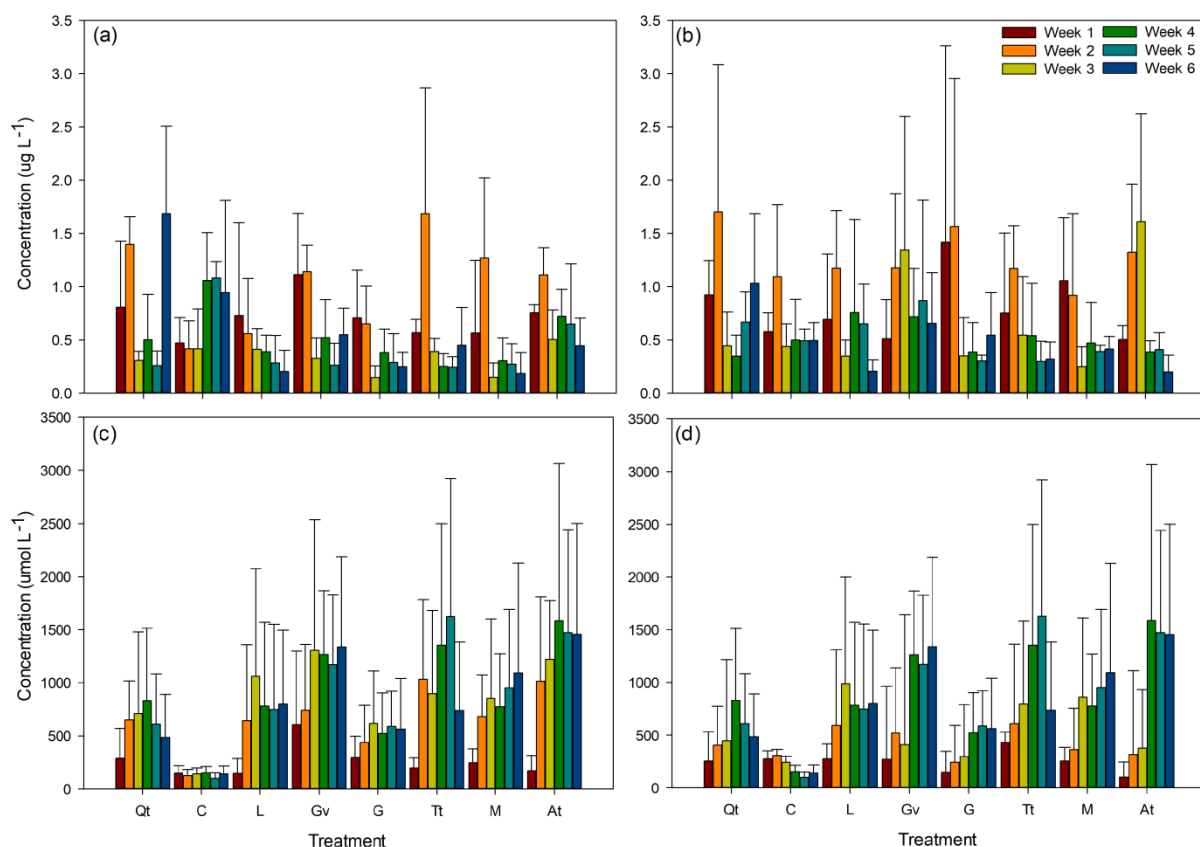
Significant differences were observed across weeks (chl–*a* – F = 9.950, *p* < 0.001; silicate – F = 22.780, *p* < 0.001), treatments (chl–*a* – F = 15.230, *p* < 0.001; silicate – F = 2.459, *p* = 0.019) and ash concentrations (chl–*a* – F = 5.856, *p* = 0.016; silicate – F = 16.718, *p* < 0.001). Significant differences were also observed across week × treatment (F = 1.753, *p* = 0.008), and week × ash (F = 2.309, *p* < 0.045) for chl–*a* concentrations, and with silicate concentration significant differences being observed for treatment × ash (F = 9.473, *p* < 0.001). Based on post–hoc analysis, significant chl–*a* concentration differences were for observed for week 1 *vs* 2 (*p* = 0.007), week

2 *vs* 3 ($p < 0.001$), 4 ($p < 0.001$), 5 ($p < 0.001$) and 6 ($p < 0.001$), with significant silicate concentration differences being observed week 1 *vs* 3 ($p < 0.001$), 4 ($p < 0.001$), 5 ($p < 0.001$) and 6 ($p = 0.005$). However, no significant chl–*a* concentration differences ($p > 0.05$) were observed for treatments, whereas significant silicate concentration differences were observed to differ for control *vs* lantana ($p = 0.001$), guava ($p < 0.001$), Transvaal milkplum ($p < 0.001$), mixed ($p = 0.001$) and apple tree ($p < 0.001$), and gum *vs* Transvaal milkplum ($p = 0.012$).

2.3.4 *Relationship between chlorophyll–a, silicate and physicochemical variables*

For the 2 g L^{-1} weight, positive and significant relationships ($p < 0.05$) were observed for the control (resistivity), guava (ORP, phosphate) and apple tree (phosphate) treatments with chl–*a* concentrations, whereas significant negative relationships ($p < 0.05$) were found for guava (silicate) and mixed (nitrite, nitrate) with chl–*a* concentration (Table S1). For the 1 g L^{-1} ash concentration, no significant relationships ($p > 0.05$) were observed for quinine tree, lantana, guava, Transvaal milk plum and apple tree treatments with chl–*a* concentration (Table S1). The 1 g L^{-1} mixed treatment had negative and significant correlations ($p < 0.05$) observed for nitrate, nitrite, pH, conductivity, TDS and salinity, with significant positive relationships observed for ORP with chl–*a* concentration. A negative and significant relationship ($p < 0.05$) was observed for chl–*a* concentration with silicate, nitrite, nitrate, conductivity and salinity in the gum treatment. For the controls, a significant negative relationship ($p < 0.05$) was observed for ORP, whereas a significant positive relationship ($p < 0.05$) was observed for nitrate, conductivity and salinity for chlorophyll–*a* concentration (Table S1).

For the 2 g L^{-1} ash treatment, lantana, gum and apple tree treatments had the most significant correlations ($p < 0.05$) observed with silicate concentrations. No significant differences ($p > 0.05$) were observed for silicate concentration with most of the physicochemical variables in treatments (Table S2). For silicate under 1 g L^{-1} ash weights, no significant relationships ($p > 0.05$) were observed for the control treatment. With lantana, all variables were significant ($p > 0.05$) (except ammonium, temperature, chl–*a*) as well as for gum (except phosphate, ammonium, ORP, pH, temperature) (Table S2).

2.4   Discussion

This study assessed the effects of native and alien leaf ash on physicochemical, silicate and chlorophyll–*a* concentrations using ash from three native (Transvaal milk plum, quinine tree, apple leaf) and three alien (lantana, guava, gum) plant species, replicating inputs from wildfires. Wildfires are an increasing problem, especially in the tropics and subtropics, due to changing climates, i.e., extreme temperatures and drought conditions, biological invasions and anthropogenic activities. In assessing the implications of ash inputs for the biomass of phytoplankton in lentic aquatic ecosystems between native and alien plant species, we did not find a clear, significant difference according to invasion history. This suggests that effects among plant species are species–specific and cannot be generalised according to invasion history, likely owing to underlying traits that influence the composition of nutrient inputs into waters. Nevertheless, the present study provides insights into the effects of wildfires on aquatic ecosystems in part of the Global South in terms of nutrient inputs and bottom–up processes.

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Different metal and nutrient concentrations for ash from each plant species were recorded; however, the ash Ca concentration was not significantly different among ash types, similar to observations by Brito et al. (2017). In comparison with other studies, such as Ulery et al. (1993), Khanna et al. (1994) and Gabet and Bookter (2011), our ash analysis yielded high concentrations of Fe and Zn, but in contrast to Liodakis et al. (2005) who observed Zn concentrations to be higher than those here. In Brazilian savannas (i.e., Cerrado biome), the ash collected had high B, Ca, K, Mg, Mn, P, S, and Zn concentrations, which were higher compared to what we recorded (Brito et al., 2017). These variations in concentrations corroborate the studies conducted by Ulery et al. (1993), Khanna et al. (1994), Demeyer et al. (2001), Liodakis et al. (2005), Gabet and Bookter (2011) and Brito et al. (2017) which highlighted that ash properties were dependent on the plant part (i.e., leaves, bark) and vegetation type.

We speculate other contributing factors that might have influenced nutrient and metal concentrations, include burning severity or temperature of combustion, which are known to influence ash properties (Bodí et al., 2014; Santín et al., 2015). However, the intensity was not measured in the current study, and this has been notably an important limitation, because plant species were possibly not subjected to consistent burning conditions given their differences in composition. After burning leaves, we observed different ash colours from each plant, with guava producing light–coloured ash compared to other plants. The apple leaf produced coarse, dark ash filled mostly with pyro–cyclic materials, indicating that the fire intensity at which the plant leaves burnt differed, as highlighted by the proposed ash colour scale (Úbeda et al., 2009). Out of 11 elements studied, six low concentrations (i.e., P, K, Ca, Mg, Cu, Zn) were recorded from apple leaf.

Overall, the ash treatments had significant differences for all the water physicochemical variables in the mesocosm, except temperature, which only differed significantly through time. Phosphates and nitrates differed significantly among treatments and ash concentration, which could have been facilitated by the P and N availability, together with the uptake rates by phytoplankton. Generally, pH increased from the 2nd week, with ash treatments recording slightly high pH compared to the controls, and in most scenarios, such increases in concentration are facilitated by the release of base cations i.e., potassium, from ash (Ulery et al., 1993; Son et al., 2015). The TDS generally peaked in week three, due to increases in water temperature in the mesocosms, allowing more solids to dissolve into the water. We have generally observed similar patterns between TDS and conductivity for all species except for the 1 g L^{-1} quinine tree and control, where the conductivity persistently increased throughout the weeks. We speculate that TDS had more influence on the conductivity by adding more ions to the mesocosm while promoting the electrical conductivity of the water.

Chlorophyll–*a* concentrations were found to generally increase a week after the addition of ash, followed by a decrease in the following weeks, however, individual patterns differed amongst treatments. We measured and recorded chl–*a* peak concentrations seven days after the introduction of ash within the system; however, it is probable that the peak concentrations temporarily peaked beyond these recordings within six days of the sampling cycle from the addition of ash, corroborating with the short period peak observations by Wang et al. (2021). An assumption is that a rapid decrease in chl–*a* concentrations after week 2 was due to depletion of ammonium and

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P in the water among the different ash treatments, following an initial 'boom' in phytoplankton, i.e., due to resource depletion. The inconsistency in weekly chl–*a* concentrations could thus be linked with disturbances to the system which promoted re–release of nutrients from ash.

Silicate concentrations generally increased throughout the weeks in both 2 g L^{-1} and 1 g L^{-1} ash treatment, with controls recording relatively low concentrations. The recorded silicate concentrations in the mesocosm may indicate that the ash treatment significantly promoted diatom growth and ultimately, their abundances (Earl and Blinn, 2003; Minshall et al., 1995). Indeed, a strong relationship between silicate and diatoms has been reported by Sumper and Kröger (2004) and Hidayat et al. (2019). This consistent increase in silicate concentrations throughout the weeks among ash treatments can be explained by the dissolution and reutilization process, whereby silica shells from diatoms are dissolved, which made silicate sufficiently available in the system while promoting vegetative cell division and formation of new valves (Paasche., 1973; Yun et al., 2018). Another possible explanation for the increase in silicate concentration concentrations could be related to the silicate in ash dissolving as the ash was added in water (Brito et al., 2017).

In the present study, ash treatment has shown numerous potential alterations within the aquatic environment, as evidenced by correlation analyses. We observed increased conductivity in contrast with baseline readings although guava, gum and Transvaal milkplum yielded slightly lower conductivity at 2 g L^{-1} compared to 1 g L^{-1}. An inverse relationship between conductivity and resistivity is notable, but the ash effect on these parameters is not significant. The mesocosms showed varying

concentrations of physicochemical parameters with increased concentration of ash input i.e., nitrate and phosphate showed a clear positive relationship with the amount of ash added into the mesocosm thus N and P concentration is expected to elevate with continuous ash input. Ammonium showed a weak relationship with increasing ash input.

While this study presents a first approach to examine the effects of ash from these alien and native plants singularly and in total combination, further work should examine the influence of broader plant combinations to better resemble variations in community composition at different invasion stages. In this context, our results could be considered to reflect the composition of ash following burning of invasive alien plants present in monoculture for the most part. Moreover, future work should examine the influence of burn intensity and the effects of different parts of plants, such as leaves and stems, in their influence on aquatic ecosystems. Additional studies could also further examine the influence of wildfire ash on different cation elements over time, whereas the present study was limited to examination in the initial ash samples before their addition to waters, albeit in relatively high concentrations.

2.5   Conclusions

The study assessed the relationships and effects of wildfire ash on water chemistry, chl–*a* (phytoplankton biomass proxy) and silicate concentrations (diatom proxy), as well as ash properties directly. It is important to highlight that the work was done in a standardised way, however, for comparing these species as a first, 'pioneering' approach to understand alien–native ash effects. A clear difference between native and alien plant ash influence on chl–*a* and silicate concentrations was not observed

amongst treatments, however, individual species exhibited varying effects on chl–*a* concentrations, but broadly similar effects on silicate concentrations. Our results suggest that ash has an influence on water chemistry i.e., elevated pH, altered nutrient concentrations and chl–*a* together with silicate concentrations, and thus the primary productivity by the aquatic ecosystems is likely to be subjected to either beneficial or detrimental effects in the events of extreme fires and deposition of ash in large quantities, with potentially mixed effects on aquatic taxa. Ash effects on the aquatic primary productivity, imposed by restructuring of the primary producers i.e., phytoplankton, are also expected to have significant impacts on higher trophic levels, such as herbivores within the affected aquatic ecosystems, which requires further examination.

CHAPTER 3: ASSESSING THE EFFECTS OF NATIVE AND ALIEN PLANT ASH ON MOSQUITO ABUNDANCE

3.1 Introduction

Wildfires have attracted increasing attention as climates change, particularly due to their direct and indirect impacts on ecosystems. Invasive alien plants have been responsible for altering fire regimes in many biomes (Brooks et al., 2004). Plant invasions are a growing concern across geographic regions and habitat types, causing both negative and positive ecological and economic impacts (Vilà et al., 2011; Hejda 2011). There are numerous negative impacts relating to these invaders, including excessive utilization of water (Calder and Dye, 2001), alteration of water quality (Chamier et al., 2012), and restructuring and displacement of native species (Hejda et al., 2011). Invasive alien plants have been, in some cases, linked with human health implications achieved through two mechanisms, i.e., production of biotoxins/allergens and provision of suitable habitat for pathogen/parasite vectors (Mack et al., 2000; Mazza et al., 2014). Invasive alien plants such as lantana (*Lantana camara*) tend to increase the frequency of wildfires by providing greater biomass which is easier to ignite than native species (Tunison et al., 2000; Bell et al., 2009; Berry, 2011).

In South Africa alone, over 200 alien plants are considered invasive, with most of them occurring within riparian environments (Chamier, 2012). Hence, this leaves the riparian vegetation, together with its helophyte communities, exposed to invader–related impacts such as wildfires (Pinto et al., 2004; Pettit., 2007). Globally, wildfires have gained much attention as one of the sources of contaminants to aquatic ecosystems, due to their associated production and deposition of foreign substances,

i.e., ash, into adjacent aquatic environments (Silva et al., 2015). Water quality in aquatic ecosystems usually decreases following wildfires (Kristensen et al., 2014; Kinoshita et al., 2016), with significant implications for the ecosystem's functionality.

We studied three widespread invasive plants in South Africa: lantana *L. camara*, gum *Eucalyptus camaldulensis,* and guava *Psidium guajava*. The records of lantana in South Africa date back to 1858, with the current infestations expected to spread over a wider range due to climate change and dispersal by birds and water (Vardies, 2012). About two million hectares were declared lantana–infested in the year 2000 (Le Maitre et al., 2000). Globally, *L. camara* poses a wide range of impacts in ecosystems it infests, for instance: elevated fire frequency and intensity (Berry, 2011), reduced water quality (River Health Programme, 2003), high water usage (Le Maitre et al., 2016), and displacement of native vegetation. Very little is known about the red river gum *E. camaldulensis* invasion history in South Africa, but its introduction dates to 1870, and it has become the most widespread invasive eucalypt amongst all species in South Africa (Hirsch, 2020), and is well established along water courses (Forsyth et al., 2004). Eucalypts including *E. camaldulensis* are speculated to have a fire risk hazard, especially crown fires (Hirsch, 2020). This eucalypt also poses allelopathic effects and displaces native vegetation (Ruwanza, 2015), alters soil physicochemical properties (Tererai, 2015), and has high water usage (Le Maitre et al., 2016). However, it is also used for numerous other benefits such as timber, firewood, (Forsyth et al., 2004). Similarly, *P. guajava* is a plant of many uses. Guava was introduced to South Africa by European settlers as a crop (Anthony, 2011). In most South African biomes, guava has been identified as the most important invasive species, particularly for its uses (Anthony, 2011), however, the negative environmental impacts of guava are not well

documented. Guava impacts include economic loss due to costs of removal (Anthony, 2011), hosting of pathogens (Mwatawala, 2006), allelopathic effects (Chapla, 2010), and displacements of native species.

Macroinvertebrates, amongst other aquatic organisms, are affected by a decrease in water quality, mainly due to the introduction of foreign substances (Xu et al., 2014), such as ash. These effects can harbor changes in species diversity, richness, and composition (Pinto et al., 2004; Rizo–Patrón et al., 2013). After a disturbance in aquatic ecosystems, macroinvertebrates may also display preferential colonization of affected areas within the ecosystem (Beckett and Miller, 1982; Vaz et al., 2014). Moreover, the recovery of streams to pre–fire conditions takes time (i.e., up to ten years) depending on the stream (Verkaik et.al., 2013) and may depend mainly on the time between fire and postfire flows together with their magnitudes (Verkaik et al., 2015).

Mosquitoes are external colonists to various waters (i.e., ovipositing eggs from the terrestrial realm), from small water containers to large water bodies (Caillouet, 2008; Medlock and Vaux, 2013), with contaminated waters being most likely to be colonised by certain mosquito species (Vonesh and Kraus, 2009; Ozeri, 2020). Water is essential for the early life stages of mosquitoes (Dale and Knight, 2008); specifically, all larvae and pupae develop in the water until the adult stage (Harbach and Besansky, 2014), whereas eggs can be deposited directly in water or land that will be flooded. Mosquitoes are mostly a risk and pest to humans and wildlife by disease transmission and nuisance biting (Jupp; 2005, Dale and Knight, 2008). However, they also have a significant ecological role in the environment. They are a prey item for other species

in food webs (Fang, 2010) and secondarily pollinate certain plants (Harbach and Besansky, 2014).

In this study, we analyzed the differences in mosquito abundances after colonization in water treated with ash from three native (i.e., quinine tree, Transvaal milk plum, and apple leaf) and three invasive alien plants (i.e., lantana, guava, and gum) plants. The plant species selected for the study usually occur near water resources in South Africa; thus, ash produced from these plants during fires is more likely to be deposited or leached into adjacent aquatic environments. This study aimed to investigate the relationship between wildfires and mosquito abundances, by assessing how ash generated from alien and native plant species with two concentrations (i.e., 1 g L^{-1} and 2 g L^{-1}) may influence the abundance of early stages of mosquitoes (i.e., eggs, larvae, and pupae) in waters. We hypothesize that ash will positively influence the abundance of mosquitoes owing to the improved habitat suitability which may attract adult mosquitoes for oviposition and benefit their development. Moreover, we posit that invasive alien plants will further promote greater abundances of vector mosquitoes than the native plant species, given the provisioning of higher biomass.

3.2   Materials and methods

3.2.1 Experimental design

The experiment was conducted at the University of Venda, Department of Geography and Environmental Sciences Atrium (–22.977550, 30.443851). The climate of the study area is classified as a humid and subtropical, with the average rainfall ranging between 400 and 800 mm and peaking between January and February. The average temperature during the warm season is just above 28°C and just below 24°C for the

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cold season; the temperatures ranged between 26°C and 30°C during the experiment. The study was conducted using 64 × 12 L buckets (⌀ 25cm, 30 cm depth). The buckets were placed and filled with 10 L filtered (63 µm mesh to remove zooplankton) river water collected from Mvudi River (–22.983544, 30.443331). The point of water collection was at the riffles; the discharge of the river was approximately 5000 L per minute. The river is relatively shallow, reaching just a few metres deep at the collection point. The river portion where water was collected and a few kilometres up and downstream can be classified as urban. In the region, wildfires usually occur at a smaller scale, often arising from agricultural fields or homesteads, but during water collection, we did not identify any fire activities. Water was collected and stored in a quarter–full 1000 L container and then transported to the departmental atrium. The container was emptied into the experimental buckets. Five grams of fertiliser (3:2:1 N:P:K ratio; Wonder Garden Care, Johannesburg) were added into the water to facilitate 'baseline' phytoplankton growth over 30 days before the start of the experiment.

Twigs with leaves were collected from three native (i.e., *R. caffra*, *E. magalismontanum*, *P. violacea)* and three invasive alien (i.e., *L. camara, P. guajava, E. camaldulensis*) plants before being sundried for 40 days in an open yard at Thohoyandou Unit C (September to October 2020). Once the leaves and twigs had dried, each plant species was separately placed inside a metal bucket, then ignited with a matchstick and allowed to burn for 50–60 minutes to produce ash. The fire intensity was not standardized across the species, but adequate in each case to produce a representative ash sample for experimentation. The fire was extinguished by covering each metal bucket with a lid. All of the ash was collected separately per

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species after it had cooled down and then placed into labelled zip lock bags to form the six individual ash treatments (i.e., per species) and an additional seventh treatment (mixed) using equal proportions of the other six individual ash treatments.

The experiment used a randomized design, with eight species treatments [i.e., 3 native, 3 alien, 1 mixed, 1 control (no ash)] × 4 replicates × 2 ash concentrations (i.e., 1 g L^{-1}, 2 g L^{-1}), and was run from 05 November 2020 to 10 December 2020. Ash at 10 g or 20 g mass was randomly added into the individual buckets, except for controls, where no ash was introduced. The ash mass was chosen to assess the different mosquito abundances under varying ash concentrations. To compensate for water loss, borehole water was used to top up the buckets to initial levels. These water additions were well–balanced among buckets.

After six weeks, each bucket was strained through a sieve (63 μm) and all the bucket contents were collected into small containers with 30 mL of 70% ethanol. Contents collected from buckets were placed under a microscope (Zeiss Stemi 2000–C) and observed between 20× and 30× magnification. Mosquitoes were counted and recorded as eggs and larvae for *Culex* spp. and *Anopheles* spp. Pupae, however, were recorded in combination for both genera as they did not exhibit prominent morphological differences. All the eggs were counted individually. Mosquito genera were identified using a mosquito morphology guide (Becker et al., 2010).

3.3   Results

We recorded colonization by two mosquito genera (*Culex* spp. and *Anopheles* spp.), with *Culex* spp. most abundant (Table 3.1). We recorded colonization by two mosquito

genera (*Culex* spp. and *Anopheles* spp.). Overall, there was no statistical significance amongst life stages of the identified genera for treatment and concentration except for pupae ($p = 0.005$) which differed amongst treatments. The pupae differed significantly in Transvaal milk plum vs control ($p = 0.032$), mixed ($p = 0.020$), quinine tree ($p = 0.012$). An analysis between plant groups was done and indicated no significant differences ($p = 0.82$).

Table 3.1: Mosquito abundances for eight level treatment; 6 plant species (apple leaf, guava, gum, lantana, quinine tree, Transvaal milk plum) + mixed + conrol under two ash concentrations (1 g L^{-1} and 2 g L^{-1}).

Treatment	*Culex* spp.		*Anopheles* spp.		Pupae
	Egg	Larvae	Egg	Larvae	(Combined)
(a) 1 g L^{-1}					
Apple leaf	53	17	0	6	5
Control	0	52	0	13	7
Guava	87	46	0	49	7
Gum	0	54	0	0	7
Lantana	141	54	0	4	11
Mixed	0	63	0	27	0
Quinine tree	0	29	12	3	0
Transvaal milkplum	0	54	0	20	50
(b) 2 g L^{-1}					
Apple leaf	343	80	0	45	12
Control	113	0	0	5	1
Guava	78	67	0	3	27
Gum	0	50	0	16	0
Lantana	107	112	0	0	14
Mixed	0	13	0	14	6
Quinine tree	0	34	0	8	1
Transvaal milkplum	105	54	0	2	10

For 1 g L^{-1}, *Anopheles* eggs were only recorded in quinine tree ash treatment, consequently, no evidence was recorded for *Anopheles* eggs statistical significance. No larval mosquitoes were recorded in gum treatment, however, this yielded significant difference in contrast to controls (p = 0.027). High abundances of *Anopheles* were recorded in guava and low abundances in quinine trees (Figure 3.1). No *Culex* eggs were recorded in gum, mixed, quinine tree ash treatments. However, high abundances were recorded in lantana, with low abundances recorded in controls. *Culex* larval mosquitoes were recorded in all treatments, with high abundances in the gum and low abundances in the apple leaf (Figure 3.1). Mixed and quinine treatments did not show the occurrence of pupal mosquitoes under 1 g L^{-1} treatments. However, milkplum recorded higher abundances in overall abundance than other treatments, while low abundances were recorded in controls (Figure 3.1), as a result, statistical significance occurred between two treatments (p = 0.044).

For 2 g L^{-1} treatments, no *Anopheles* spp. were eggs recorded, this count variable was excluded from further analysis. However, larvae were recorded in all treatments except for lantana, with high abundances recorded in apple leaf for both genera (Figure 3.1), a clear statistical significance was observed between apple leaf vs controls (p = 0.008) and lantana vs controls (p = 0.027). Apple leaf caused the highest *Culex* egg abundances at this concentration (p = 0.022), with no eggs found in gum, mixed, or quinine groups, thus no evidence was found. *Culex* larvae were recorded in all the treatments, with lantana recording high abundance and mixed ash recording low abundance (Figure 3.1), resulting in clear statistical significance observed between lantana and controls (p < 0.001). Pupae were recorded in all 2 g

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L^{-1} treatments, with increased abundances in guava and low quantities in the quinine tree (Figure 3.1).
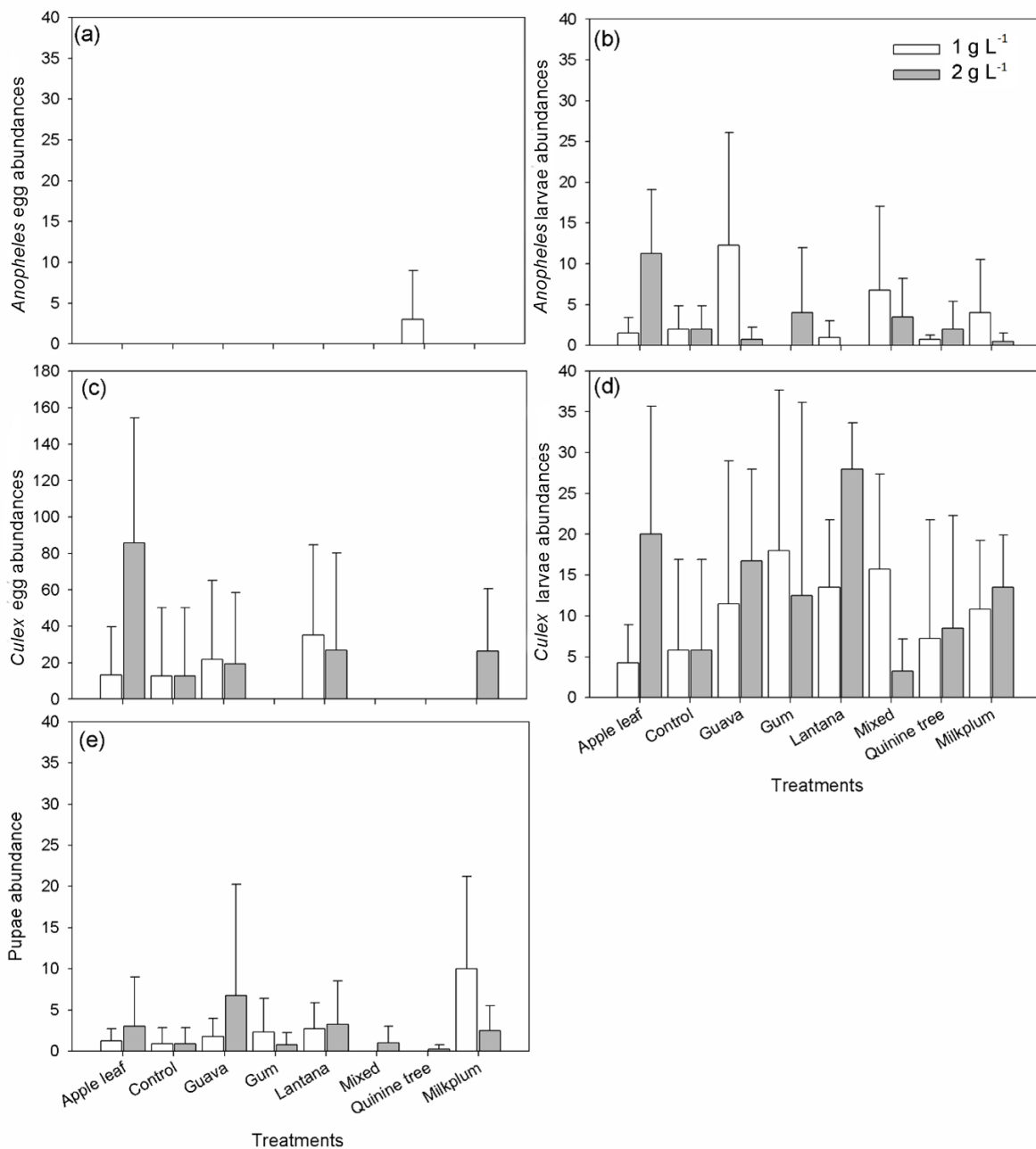
Figure 3.1. Mean (±SD) *Anopheles* spp. eggs (*a*) and larvae (*b*); *Culex* spp. eggs (*c*) and larvae (*d*), and pupae (*e*) abundances among the different experimental

treatments. Abbreviations: Milkplum – Transvaal milk plum; Native: quinine tree, Transvaal milk plum, apple leaf; Alien: lantana, guava, gum.

3.4 Discussion

Plant invasions are considered a severe concern to ecosystems due to their numerous adverse impacts such as alteration of fire regimes globally and potential deposition of ash into aquatic environments. In the current study, we assessed how different ash types generated from alien plants compared to native plants with two concentrations (1 g L $^{-1}$ and 2 g L^{-1}) might influence the abundances of early stages of mosquitoes in the water. We also assessed the effects of ash concentration on their abundance after colonization.

Overall, no clear pattern was demonstrated between native and alien species' influence on the mosquito abundances, similarly, no clear pattern was demonstrated by the ash concentration choices for this study. We strongly believe that a clear pattern could have been observed at a certain point with the use of a wide range of concentrations, numerous plant species, and waters of various regions in the mesocosm.

The variations in *Culex* eggs caused by apple leaf could have been coupled by habitat suitability i.e., patch size (Bohenek et al., 2017) and preferences (Lampman, 1996), attracting more female *Culex* mosquitoes for oviposition. Alternatively, there is a high possibility that the overall abundance may have been influenced by attractants such as nutrients and food availability in the early stages, but these require further elucidation. The *Culex* mosquitoes are usually the hastiest to colonize waters

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(Williams et al., 1993), which may grant them a chance to successfully colonize newly established or disturbed aquatic environments. In the current study, we recorded high abundances of *Culex* mosquitoes compared to the *Anopheles*, and thus concluded that the environmental conditions i.e., temperature, and the entire water chemistry (Kinga et al., 2022) greatly attracted *Culex* mosquitoes over *Anopheles* mosquitoes. However, in other studies conducted in Sudan (Seufi, 2010), South Africa (Munhenga, 2014), and Tanzania (Emidi et al., 2017), high abundances of *Anopheles* mosquitoes have been recorded during the same period (November–December), probably because they are not container breeders and possibly prefer cleaner water (Munga et.al., 2005) compared to the *Culex* mosquitoes that are usually found in high abundances in waters with impurities (Medlock and Vaux, 2014), and for that reason, we would have expected to record more *Anopheles* mosquitoes in controls. Nonetheless, the densities for *Anophele*s mosquitoes are known to be seasonal across regions and could relate to their oviposition of eggs singularly compared to in batches as in *Culex*.

There were no significant effects recorded among ash groups (treatments). Nonetheless, the *Culex* mosquitoes generally increased in abundance with increased ash concentration; in apple leaf, guava, and lantana, this may indicate a potential risk to public health in cases where ash is leached into water bodies in large quantities, following the evidence that *Culex* mosquitoes have been reported to be one of the primary vectors of the rift valley fever virus (Seufi, 2010), however, this needs further studies with a wide range of concentrations. Although not assessed in the current study, it cannot be entirely ruled out that the ash could have exhibited insecticidal effects at some level probably due to plant extracts (Kamaraj, 2011). Indeed, some

plant extracts have been linked with insecticidal effects on vector mosquitoes (Sakthivadive, 2008; Niroumand, 2016), moreover, such effects may vary depending on the plant part i.e., leaves, stem or flowers (Alfaki, 2015), thus we could have observed more patterns in mosquito abundances for similar species with ash produced from different plant parts. In the three life stages considered (i.e., egg, larvae and pupae), the pupal stage is usually the shortest; there is a possibility that the sampling period might have missed the short window before transformation into adult mosquitoes. Moreover, we had expected to record *Aedes* spp. as they are commonly found in the experimental area and are familiar colonists in container–style habitats (Jupp, 2005). Their absence was likely because they oviposit above the water line, with the water level not increasing over the experiment and promoting hatching.

There is uncertainty regarding the tolerable ash concentrations of the identified mosquitoes. Similarly, there are also uncertainties concerning the long–term effects of ash deposited into aquatic ecosystems on the abundance of mosquitoes. However, the ecological drivers of mosquito population in colonizing successfully are likely to depend on local conditions rather than the broad regional scale, and the effect of ash on mosquito abundances may vary from plant to plant probably owing to plant ash chemical composition rather than history of occurrence and distribution.

3.5 Conclusion

The study assessed the effects of two ash concentrations on mosquito abundances using six plant species, three of which are native and three are invasive and alien. We recorded higher numbers of *Culex* spp. in contrast with *Anopheles* spp., probably owing to habitat selectivity and egg–laying behavior. The possibility of season playing

a role cannot be ruled out either. Generally, the ash generated from the selected species had no notable effects on the abundance of mosquitoes. Although not statistically significant, ash concentration seemingly played a role in the abundance of *Culex* spp. as the abundance slightly increased with the increasing concentration, indicating a potential risk of vector mosquitoes that may, in turn cause implications to human health. Nonetheless, further study is required to assess the ash elements that are likely to attract or repel mosquitoes and exploring different water sources or habitat types.

3.5.1 *Statistical analysis*

Generalized Linear Models (Poisson log–linear regression model) were used to analyze the count variables against two factors, i.e., treatment (6 species + mixed + control) and concentration (1 g L^{-1} + 2 g L^{-1}) for main effects, and their interaction. The count variables were grouped by genera and life stage, and pupae were analyzed together (i.e., for both genera). Therefore, five models were fit in total (2 species × 2 life stages + pupae). Type I sum of squares was used for deviance analysis and assessment of statistically clear levels of the main effects at $p < 0.05$. Estimated Marginal Means for factors and their interactions were computed following Least Significant Difference for pairwise comparison with Tukey adjustment. Counts were similarly analyzed coarsely against another factor, i.e., species type (alien + native) with other factors pooled together. All statistical analysis was done using SPSS, version 24 (IBM Corp. 2016).

CHAPTER 4: GENERAL SYNTHESIS

4.1 General discussion

Wildfires coupled with alien plant invasions have been intensively studied in most bioregions (Tunison et al., 2000; Brooks et al., 2004). Plant invasions are marked as one of the growing concerns worldwide mainly due to their ecological impacts. Amongst other impacts, alien invasive plants tend to alter fire regimes (Brooks et al., 2004) with subsequent effects on the adjacent environments. Aquatic environments are prone to accumulation of ash generated from wildfires, deposited by either wind or water. The deposition of ash into aquatic ecosystems is expected to have an

influence on the bottom–up processes including phytoplankton biomass and mosquito abundance.

The purpose of the study was to assess the effects of wildfire ash from native and alien plants on phytoplankton biomass and on mosquito abundance. The key finding of this study is that ash effects on phytoplankton vary with individual species and ash concentration, similarly with mosquito abundance. Our ash properties varied greatly with individual species; we recorded different element levels in individual species see (CHAPTER 2: **EFFECTS OF WILDFIRE ASH FROM NATIVE AND ALIEN PLANTS ON PHYTOPLANKTON BIOMASS**). In assessing the effects of ash in water chemistry we found that our ash had notable effect on water chemistry, however, the effect varied with species except for nitrate with generally increased over time.

4.2   Conclusions and recommendations

The study indicated that the concentration of ash chemical properties varies with individual species, however, we did not find clear patterns between alien and invasive species in ash properties. We recommend that future studies also assess a wide range of plant species from distinctively varying bioregions. We noted the effects of ash on the water chemistry does vary with species and ash concentration, we also indicated which physicochemical parameters were highly influenced by ash.

The study found relatively high abundance of *Culex* mosquitoes in contrast to the *Anopheles*. We speculated the effect of season in the mosquito abundance; however, we recommend further studies to consider the seasonal variations in mosquito abundances. Although not statistically significant, ash concentration seemingly played

a role in the abundance of *Culex* spp. as the abundance slightly increased with the increasing concentration, indicating a potential risk of vector mosquitoes that may, in turn cause implications to human health. Nonetheless, further study is required to assess the ash elements that are likely to attract or repel mosquitoes and exploring different water sources or habitat types.

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Table S1. The Pearson correlation analysis for chlorophyll–*a* concentration and physicochemical variables among the different ash weights

Variables	Quinine		Control		Lantana		Guava		Gum		Transvaal milkplum		Mixed		Apple tree	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
20 g																
Phosphate	0.17	0.458	–0.19	0.414	–0.33	0.144	**0.41**	**0.050**	0.21	0.364	0.05	0.844	0.26	0.218	**0.49**	**0.027**
Silicate	–0.09	0.683	–0.18	0.446	0.09	0.684	**–0.56**	**0.005**	–0.19	0.411	0.12	0.614	–0.20	0.344	–0.28	0.235
Nitrate	0.18	0.436	–0.38	0.092	–0.15	0.520	–0.22	0.305	–0.41	0.066	–0.36	0.107	**–0.45**	**0.026**	0.07	0.771
Nitrite	0.30	0.188	–0.29	0.208	–0.10	0.663	–0.23	0.286	–0.30	0.186	–0.29	0.196	**–0.45**	**0.028**	0.04	0.855
Ammonium	–0.03	0.911	–0.29	0.197	–0.10	0.661	0.07	0.739	0.21	0.359	0.05	0.814	–0.35	0.097	0.29	0.217
pH	–0.02	0.933	–0.06	0.797	–0.32	0.129	–0.51	0.011	–0.31	0.139	0.13	0.530	–0.12	0.589	–0.21	0.331
TDS	–0.05	0.822	–0.35	0.095	0.06	0.796	–0.25	0.236	–0.12	0.583	0.09	0.676	–0.20	0.355	–0.34	0.104
Conductivity	–0.07	0.738	–0.33	0.112	0.06	0.787	–0.40	0.053	0.06	0.796	0.21	0.320	–0.05	0.824	–0.18	0.397
ORP	–0.03	0.872	0.05	0.831	0.28	0.178	**0.52**	**0.010**	0.34	0.103	–0.11	0.599	0.14	0.520	0.24	0.261
Resistivity	0.01	0.959	**0.48**	**0.017**	–0.10	0.652	0.23	0.278	0.03	0.904	–0.16	0.457	0.15	0.481	0.30	0.156
Temperature	0.01	0.950	–0.22	0.299	0.06	0.779	–0.08	0.715	–0.21	0.328	–0.21	0.326	–0.28	0.186	–0.29	0.174

Salinity	−0.07	0.732	−0.32	0.125	0.06	0.783	−0.26	0.223	0.05	0.802	0.21	0.315	−0.01	0.960	−0.17	0.434

10 g

Phosphate	−0.12	0.603	−0.32	0.123	0.00	0.987	0.38	0.064	0.06	0.840	0.28	0.226	0.28	0.229	0.28	0.226
Silicate	−0.07	0.754	0.05	0.813	0.06	0.811	−0.24	0.255	**−0.69**	**0.005**	−0.01	0.972	−0.34	0.149	−0.01	0.972
Nitrate	−0.17	0.450	0.36	0.081	−0.18	0.426	−0.24	0.267	**−0.62**	**0.013**	−0.37	0.106	**−0.48**	**0.033**	−0.37	0.106
Nitrite	−0.12	0.597	**0.65**	**0.001**	−0.13	0.577	−0.30	0.153	**−0.55**	**0.034**	−0.28	0.230	**−0.50**	**0.024**	−0.28	0.230
Ammonium	−0.05	0.813	0.04	0.856	−0.04	0.847	0.08	0.720	−0.42	0.116	−0.14	0.566	−0.30	0.194	−0.14	0.566
pH	−0.10	0.643	0.34	0.099	−0.25	0.247	−0.19	0.380	−0.27	0.279	−0.19	0.375	**−0.53**	**0.008**	−0.19	0.375
TDS	−0.23	0.274	0.17	0.419	−0.28	0.183	0.25	0.236	−0.45	0.063	0.10	0.655	**−0.41**	**0.045**	0.10	0.655
Conductivity	−0.05	0.809	**0.56**	**0.004**	−0.14	0.526	0.28	0.180	**−0.64**	**0.004**	−0.06	0.790	**−0.52**	**0.009**	−0.06	0.790
ORP	0.10	0.649	**−0.60**	**0.002**	0.13	0.544	0.25	0.234	0.27	0.274	0.15	0.486	**0.51**	**0.012**	0.15	0.486
Resistivity	0.22	0.292	−0.24	0.260	0.27	0.197	−0.24	0.249	0.46	0.052	−0.04	0.858	0.02	0.934	−0.04	0.858
Temperature	−0.26	0.224	−0.28	0.183	−0.33	0.123	0.07	0.758	−0.09	0.729	0.16	0.456	−0.17	0.431	0.16	0.456
Salinity	−0.15	0.474	**0.54**	**0.007**	−0.14	0.504	0.28	0.189	**−0.62**	**0.006**	−0.03	0.889	**−0.47**	**0.020**	−0.03	0.889

Table S2. The Pearson correlation analysis for silicate concentration with physicochemical variables among the different ash weights

Variables	Quinine		Control		Lantana		Guava		Gum		Transvaal milkplum		Mixed		Apple tree	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
20 g																
Phosphate	**−0.63**	**0.002**	0.10	0.667	**−0.50**	**0.021**	**−0.63**	**0.001**	**−0.74**	**<0.001**	−0.26	0.250	**−0.52**	**0.009**	**−0.49**	**0.028**
Nitrate	−0.05	0.841	0.34	0.135	**0.57**	**0.006**	0.13	0.563	**0.61**	**0.003**	**0.55**	**0.009**	**0.54**	**0.007**	0.10	0.668
Nitrite	0.05	0.821	**0.55**	**0.010**	**0.72**	**<0.001**	0.16	0.459	**0.44**	**0.045**	**0.64**	**0.002**	**0.56**	**0.005**	0.10	0.689
Ammonium	−0.23	0.320	−0.02	0.923	**−0.78**	**<0.001**	−0.13	0.541	**−0.54**	**0.011**	0.40	0.073	−0.13	0.548	**−0.51**	**0.022**
pH	0.16	0.488	−0.05	0.814	0.38	0.091	0.29	0.185	0.14	0.535	0.34	0.132	0.36	0.087	**0.50**	**0.023**
TDS	0.16	0.481	−0.06	0.802	0.40	0.070	0.01	0.956	**0.45**	**0.042**	0.32	0.158	−0.17	0.424	0.25	0.282
Conductivity	0.19	0.421	0.08	0.732	**0.56**	**0.008**	0.02	0.920	**0.69**	**0.001**	**0.47**	**0.032**	−0.25	0.241	**0.55**	**0.012**
ORP	−0.17	0.459	0.00	0.987	−0.35	0.124	−0.30	0.160	−0.11	0.621	−0.35	0.116	−0.35	0.095	**−0.52**	**0.020**
Resistivity	−0.12	0.601	0.08	0.743	**−0.44**	**0.044**	−0.02	0.940	**−0.46**	**0.035**	−0.33	0.140	0.20	0.359	−0.36	0.120
Temperature	0.01	0.963	−0.22	0.332	0.09	0.686	0.07	0.753	0.01	0.980	−0.27	0.237	−0.05	0.800	−0.10	0.688
Salinity	0.25	0.268	0.07	0.760	**0.58**	**0.006**	−0.05	0.829	**0.67**	**0.001**	**0.47**	**0.031**	−0.28	0.185	**0.53**	**0.016**
Chl–*a*	−0.09	0.683	−0.18	0.446	0.09	0.684	**−0.56**	**0.005**	−0.19	0.411	0.12	0.614	−0.20	0.344	−0.28	0.235
10 g																
Phosphate	**−0.78**	**<0.001**	−0.16	0.460	**−0.80**	**<0.001**	**−0.42**	**0.040**	0.03	0.922	−0.04	0.861	**−0.59**	**0.007**	−0.04	0.861

Nitrate	−0.08	0.738	0.18	0.387	**0.44**	**0.045**	−0.08	0.720	**0.63**	**0.012**	**0.67**	**0.001**	0.29	0.223	**0.67**	**0.001**
Nitrite	0.09	0.684	0.13	0.557	**0.46**	**0.037**	−0.02	0.934	**0.65**	**0.009**	**0.59**	**0.006**	0.38	0.099	**0.59**	**0.006**
Ammonium	**−0.69**	**<0.001**	0.36	0.084	0.39	0.082	0.39	0.062	0.42	0.123	0.18	0.450	0.32	0.173	0.18	0.450
pH	0.21	0.337	−0.22	0.308	**0.43**	**0.051**	0.25	0.247	0.40	0.142	**0.73**	**<0.001**	0.36	0.120	**0.73**	**<0.001**
TDS	0.18	0.427	0.25	0.232	**0.60**	**0.004**	0.17	0.424	**0.59**	**0.022**	0.16	0.512	**0.63**	**0.003**	0.16	0.512
Conductivity	0.24	0.290	0.04	0.868	**0.71**	**<0.001**	0.31	0.140	**0.86**	**<0.001**	0.22	0.348	**0.85**	**<0.001**	0.22	0.348
ORP	−0.15	0.497	−0.10	0.652	**−0.58**	**0.005**	−0.25	0.235	−0.40	0.139	**−0.62**	**0.003**	−0.37	0.110	**−0.62**	**0.003**
Resistivity	−0.19	0.390	−0.17	0.435	**−0.58**	**0.006**	−0.19	0.372	**−0.61**	**0.016**	−0.16	0.494	−0.24	0.308	−0.16	0.494
Temperature	0.02	0.913	0.25	0.248	0.08	0.735	−0.13	0.555	0.04	0.887	0.02	0.941	0.08	0.743	0.02	0.941
Salinity	0.21	0.359	0.12	0.592	**0.71**	**<0.001**	0.29	0.165	**0.84**	**<0.001**	0.21	0.383	**0.87**	**<0.001**	0.21	0.383
Chl–a	−0.07	0.754	0.05	0.813	0.06	0.811	−0.24	0.255	**−0.69**	**0.005**	−0.01	0.972	−0.34	0.149	−0.01	0.972