

AN ABSTRACT OF THE THESIS OF

Megan R. Drinnan for the degree of Master of Science in Geology presented on December 3, 2013

Title: Biotic Alteration of Oceanic Basalt Glass

Abstract approved:

Martin R. Fisk

Anita L. Grunder

The subsurface microbial biosphere in the igneous oceanic crust has implications for global geochemical cycling, early life on Earth, and the search for life on Mars. Microscopic evidence of this subsurface microbial ecosystem includes biotic alteration textures associated with basaltic glass. The exact conditions in the basaltic layer that make this a viable ecosystem remain unknown. Geologic investigations rely on the principle that the present is the key to the past and therefore the conditions evident in rocks today can be used to elucidate conditions occurring over time. The application of this principle to basaltic rocks sampled from the modern ocean containing biotic alteration textures is the primary objective of this study. This study represents a global investigation into the relationship between the morphology of biotic alteration and its abundance in the ocean crust and the environmental conditions associated with the host rocks. The Ocean Drilling Program and the Deep Sea Drilling program provided subseafloor basaltic glass samples used in this study. The unique morphologies of the biotic alteration textures present were identified using a classification system and the abundance of biotic alteration was estimated as a percent of the total alteration present in the samples. Nine distinctive textures were selected for correlation with five environmental parameters including sample age, sample depth in basalt, overlying sediment thickness, temperature, and secondary mineralogy. These parameters served as proxies for the environment associated with biotic alteration such as oxidizing conditions,

fluid flow, pH, and temperature tolerances for microbial growth. Preliminary results indicated the abundance and the frequency of biotic alteration textures varied with the environmental conditions. Statistical analyses were conducted to assess the strength and direction of the variation between the biotic alteration and environmental parameters. I found that several biotic alteration textures and biotic alteration abundance had statistically significant correlations with sample age, sample depth into basalt, temperature, and some secondary minerals. The seafloor biosphere is a complex environment influenced by many variables. This investigation into how the evidence of biotic alteration preserved in basaltic glass varies with the environmental conditions represents a step towards understanding the dynamics of the ecosystem in the ocean crust.

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Biotic Alteration of Oceanic Basalt Glass

by
Megan R. Drinnan

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APPROVED:

Major Professor, representing Geology

Dean of the College of Earth, Ocean, and Atmospheric Sciences

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Megan R. Drinnan, Author

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Biotic Alteration of Oceanic Basalt Glass

1. INTRODUCTION

The ubiquitous nature of microscopic textures (Figure 1) within basaltic glass from the ocean floor has generated many questions about their formation (Bach and Edwards, 2003; Fisk and McLoughlin, 2013; Fisk et al., 1998; Furnes et al., 2001a; Furnes et al., 2008; Furnes and Staudigel, 1999; Heberling et al., 2010; Josef, 2006; Knowles et al., 2012, Knowles et al., 2013; McLoughlin et al., 2010b; Morgenstein, 1969; Orcutt et al., 2011; Santelli et al., 2008; Schrenk et al., 2009; Staudigel et al., 2009; Staudigel et al., 2006; Storrie-Lombardi and Fisk, 2004; Thorseth et al., 2001; Thorseth et al., 1998; Thorseth et al., 1995b; Walton 2007, 2008). The textures have been observed in basalt samples from on the seafloor collected by submersibles and through dredging. Examples have also been observed in basaltic glass samples from the subsurface that are obtained by the Deep Sea Drilling Program (DSDP), the Ocean Drilling Program (ODP), and the Integrated Ocean Drilling Program (IODP). For several decades the characteristics, distribution, and composition of the textures have been investigated and the exact process by which they are created remains unresolved.

One hypothesis suggests that biotic activity is a plausible mechanism producing the textures (Furnes et al., 2008, 2002; McLoughlin et al., 2009; Staudigel et al., 2008; Storrie-Lombardi and Fisk, 2004; Torsvik et al., 1998; Thorseth et al., 1992). This hypothesis has been proposed for several reasons including the resemblance of the form and size of some of the textures to biotic forms such as for example, coccoid and rod shaped cells, and filamentous structures observed in biofilm encrusting subseafloor glass (Thorseth et al., 2001). The textures also typically originate from fractures and other places where seawater, a potential nutrient source (Edwards et al., 2012; Konhauser, 2007), is in contact with the rock (Fisk et al., 1998; Furnes et al., 2008; Konhauser, 2007) (Figure 1). This resemblance to biotic structures has contributed to some authors' hypotheses that they are trace fossils (Fliegel et al., 2010; McLoughlin et al., 2010a). Other forms of chemical and experimental evidence for a biotic mechanism are presented later in this study. Because of this body of evidence the textures are called biotic in this study.

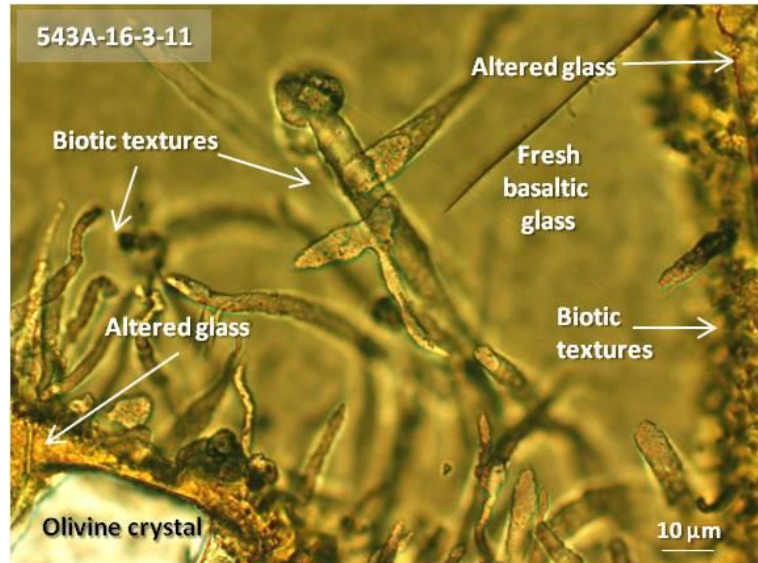


Figure 1. Photomicrograph of biotic alteration found in oceanic basalt glass.

The light brown, matrix material is basaltic glass and the orange material surrounding the olivine grain is palagonite. The tunnels pictured have oval cross sections. As they originate from the edge of the olivine crystal in the lower left and extend into the fresh basaltic glass they curve slightly. On the right edge of the image the biotic textures originate from a fracture that is outside and parallel to the edge of the photomicrograph. Although the tunnels appear to overlap, they are actually visible in three dimensions within the transparent glass. The third dimension is also visible in the tunnels that are partially in focus and partially out of focus depending on their orientation relative to the viewer. This 83 Ma sample is from the abyssal plain in the Atlantic Ocean.

Sample number is explained as follows: 543A refers to the drilling site, the core number is 16; the core section is 3, and the 11 indicates the centimeters from the top of the core section.

In terrestrial environments, microbially mediated textures that are similar to the ones in this study are frequently observed within natural and manufactured glasses (Aouad et al., 2006; Brehm et al., 2005; Drewello and Weissmann, 1997). The similarity between the terrestrial textures and the basaltic glass textures has led to the suggestion that analogous microbial processes create the marine textures also. To date however, the mechanism by which these textures form has not been replicated in laboratory experiments and thus the causal link between microbial activity and the textures in basaltic glass remains unestablished (Furnes et al., 2008; Knowles et al., 2012, Knowles et al., 2013).

Understanding the textures has implications for our understanding of biogeochemical cycling in the ocean, our knowledge of early Earth's ancient conditions and habitat, and the search for life on Mars. Biogeochemical cycles are influenced by the mass and energy flux between the subsurface and the open ocean and microbial mediation could influence this flux (Alt and Honnorez, 1984; Alt et al., 1986; Alt and Mata, 2000; Bach and Edwards, 2003; Bach, et al., 2003; Mottl et al., 2003; Orcutt et al., 2011; Staudigel and Hart, 1982). The conditions present on early Earth could be clarified by understanding the basaltic glass textures because textures bearing close resemblance to these have been found in ~3.4-3.5 Ga meta-volcanic glass (Banerjee et al., 2007, 2006; Schopf, 1993). If the textures are biotic, then the meta-volcanic examples could also be the oldest evidence for life on Earth. Observations of a Martian meteorite containing textures similar to these raise the question of whether the same process or a similar mechanism produces the Martian textures (Fisk et al., 2006).

Understanding these textures has implications that extend beyond the natural world since microbial degradation affects the longevity and integrity of manufactured glass used for building materials, and other structures (Aouad et al., 2006; Crovisier et al., 1987; Drewell and Weissmann, 1997). Manufactured glass that is vulnerable to microbial degradation is also used to store and isolate radioactive and other toxic waste (Donald, 2010).

1.2 Environmental setting

1.2.1 The ocean crust

The oceanic crust is comprised of three layers (Karson et al., 2002; Schrenk et al., 2009; White et al., 1992). Pelagic sediments form layer 1, layer 2a is composed of basaltic rocks, and layer 2b is composed of intrusive sheeted basalt dikes. Layer 3 is a combination of gabbroic rocks and ultramafic cumulates (Figure 2).

The basaltic rocks that make up layer 2a are pillow basalts, lava flows, and volcanic breccias. Basalt glass is a highly viscous metastable liquid that contains microlites or skeletal crystals, and varioles or quench textures (Furnes et al., 2008). It forms when lavas erupt onto the seafloor along mid-ocean ridges, on seamounts, back arc basin rifts, and anywhere subaerial eruptions flow into water. When lava comes into contact with cold seawater the rapid, cooling, or quenching of the lava produces the amorphous basalt glass. The biotic textures are located within volcanic glass of layer 2a (Figure 2). Volcanic glass makes up about 20% of layer 2a, occurring in the rinds of pillow lavas and as fragmented basalt or hyaloclastites (Alt and Teagle, 2003; Bouška et al., 1993; Staudigel and Hart, 1982).

Three major processes that affect the ocean crust are the transfer of heat from the Earth's interior, the circulation of fluids, and the interaction between fluids and rock in the permeable basalt layer (Figure 2). All three of these processes occur as a function of age and a function of lateral distance of the crust from the spreading axes.

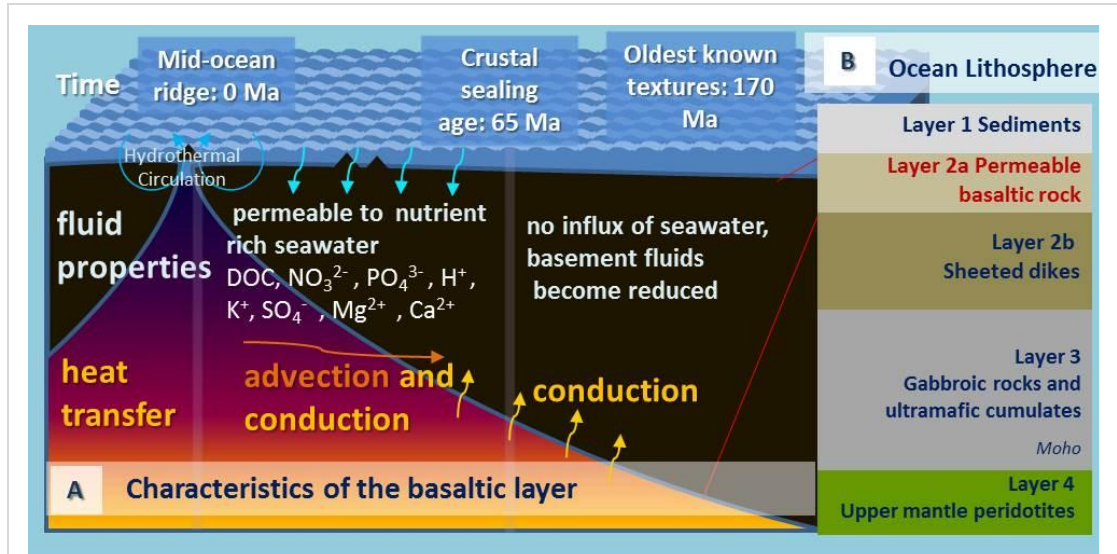


Figure 2. Schematic diagram of ocean lithosphere processes through time.

(A) The basaltic layer 2a of the ocean crust is characterized by temporally controlled temperature (2-1200°C) gradients that induce fluid circulation (Stein and Stein, 1994; Mottl, 2003). During the first 0 to 1 Ma hydrothermal circulation occurs near the ridge axis (Becker and Fisher, 2000; Fisher and Becker, 2000; Mottl, 2003; Stein and Stein, 1994). From about 1 Ma to 65 Ma advection and conduction of heat generates pressure gradients that induce vigorous basement circulation in the permeable basaltic layer. Seawater moves nutrients to the subsurface during this time. From about 65 Ma to 160 Ma the crust begins to “seal” as mineral deposition in pore space inhibits permeability. Dissolved organic carbon (DOC) and other nutrients circulate through the subsurface, however, no new material from the open ocean is introduced.

(B) Layer 2a contains volcanic glass in basaltic pillow lavas, lava flows, and breccias. Living microbial systems have been found in layer 2a (e.g. Lever et al., 2013; Lysnes et al., 2004). In addition they have also been found in layer 1 (e.g., Parkes, 1994; Parkes et al., 2000) and layer 3 (e.g., Mason et al., 2010).

Modified from Alt and Honnorez, 1984; Edwards et al., 2012; Fisher, 1998; Fisher and Becker, 2000; Fisk et al., 1999; Becker and Fisher 2000; Karson, 2002; Orcutt et al., 2011; Staudigel et al., 1984, 1984; Stein and Stein, 1994).

1.2.2 Heat flow

Heat flow from hydrothermal activity is a major process that affects the ocean crust (Elderfield and Schulz, 1996; Mottl, 1993; Stein et al., 1995; Stein & Stein 1994). The estimated 10×10^{12} W of oceanic crustal heat flow can be divided into three temporal regimes. In the first regime near the seafloor spreading axes for crustal ages of 0-1 Ma, 2 to 4×10^{12} W of heat flow occurs due to convection and advection of heat from magmatic and volcanic activity (Elderfield and Schulz, 1996; Fisher and Becker, 2000; Mottl, 1993; Stein and Stein; 1994; Stein et al., 1995). The second regime is associated with the ridge flanks, and includes crustal ages between 1-65 Ma (Figure 2). Within the ridge flank regime, 7×10^{12} W of heat advection and conduction transfers heat away from the crust. Heat flow in crust that is greater than 65 Ma, in the third regime, remains relatively constant and heat is transferred primarily by conduction, in contrast to advection and conduction on the ridge flanks.

The three differing heat flow regimes can be explained by crustal sealing when secondary mineral precipitation in void spaces reduces fluid circulation (Stein and Stein, 1994; Fisher, 1998). The crustal sealing model arose from the observation that in crust younger than 65 Ma observed heat flow values are less than those predicted by purely conductive models. Fluid circulation during this time period, <65 Ma, mediates the transfer of heat away from the mantle, lowering the heat flow values. After 65 Ma the difference between observed and predicted heat flow values dissipates because permeability and fluid flow is reduced by mineral deposition (Figure 2).

1.2.3 Fluid flow

Fluid flow is a major process that affects the ocean crust. Seawater flux through the crust occurs on such a large scale it has been proposed that the ocean does not stop at the seafloor but instead extends into the subsurface aquifer (Tunnicliffe, 2003). The oceanic crust holds an estimated 1.35×10^{18} m³ of water, or 2% of the total ocean volume, at any given time (Edwards et al., 2012; Emerson and Hedges, 2008; Orcutt et al., 2011). The entire volume of the ocean circulates through this aquifer approximately every 200,000

years (Fisher and Becker, 2000; Johnson and Prius, 2003). Seawater primarily enters the crust at seafloor spreading ridges, seamounts and other basement outcrops. Temperature driven pressure gradients cause the circulation of fluids through the basaltic layer for up to tens of millions of years (Johnson and Prius, 2003; Mottl, 1993). The large volume of seawater circulation decreases with crustal age and depth because the deposition of minerals in open space reduces permeability (Becker and Fisher, 2000; Fisher and Becker, 2000; Stein and Stein, 1995)

The ocean covers about 70% of the earth's surface and contains ions in constant ratios and other nutrients. The major ions that occur in constant ratios include sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chlorine (Cl^-), sulfate (SO_4^{2-}), bicarbonate (HCO_3^-) (Emerson and Hedges, 2008; Stewart, 2005). Of these major ions, K^+ , Mg^{2+} , Ca^{2+} , SO_4^{2-} , are considered necessary nutrients for life (Munn, 2003; Orcutt et al., 2011). Other necessary nutrients in seawater occur in variable amounts. These are dissolved organic carbon (DOC), nitrate (NO_3^-), and phosphate (PO_4^{3-}). The latter two nutrients, PO_4^{3-} and NO_3^- , are necessary to build DNA (Konhauser, 2007). Seawater contains the necessary ingredients to support microbial life (Figure 2).

The driving metabolic processes for life in seawater include photosynthesis and chemosynthesis through organic matter or other inorganic compounds (Munn, 2003). Photosynthesis in the ocean is limited to the photic zone in the upper 150 to 200 meters of water. In the rest of the water column, the aphotic zone, chemosynthesis is the dominant metabolic process (Munn, 2003). Below the photic zone life is supported by the oxidation of organic matter. The oxidation of organic matter by oxygen is progressively limited in deeper waters because of respiration and oxidation of organic carbon that occur in the shallower reaches of the water column. Deeper in the water column and in the subsurface, metabolically useful compounds in seawater for organisms include sulfate (SO_4^{2-}) and nitrate (NO_3^-) (Bach and Edwards, 2003; Emerson and Hedges, 2008; Orcutt et al., 2011; Stewart, 2005). There are between 10^6 and 10^8 microbial cells living in every 1 milliliter of seawater. Seawater is consequently a viable

transport mechanism for microbes and nutrients into the oceanic subsurface (Hoppe, 1976; Stevenson, 1978).

Essential nutrients and electrochemical concentration gradients resulting from seawater circulation in the subsurface are capable of supporting ecosystems (Bach and Edwards, 2003; Parkes et al., 2000). The circulation of seawater into the subsurface helps to support marine subsurface communities that are estimated to account for 5-15% of Earth's total biomass (Orcutt et al., 2011).

1.2.4 Water rock interactions

Water-rock interactions alter subseafloor basaltic rocks, and during this process the fluids themselves become altered (Figure 2) (Alt and Honnorez, 1984; Alt and Teagle, 2003; Bach et al., 2003; Benzerara et al., 2007; Bouška et al., 1993; Furnes et al., 2001a; Jakobsson and Moore 1986; Stroncick and Schmincke, 2002). Clays, zeolites, carbonates, and oxyhydroxides are deposited in subseafloor pore spaces, fractures, and voids typically at fluid temperatures that are less than 150°C (Alt and Honnorez, 1984; Stroncick and Schmincke 2002). Mineral deposition in open space will eventually reduce permeability and as a result mass and heat exchange between the subseafloor and the overlying water column. The crust eventually seals preventing further water-rock interactions.

Volcanic glass alteration

Abiotic basaltic glass alteration in aqueous environments begins with the formation of palagonite. Palagonite (Figure 1 and Figure 3) in this study refers to the hydration and dissolution of glass initially producing palagonite, phyllosilicates, and oxyhydroxides (Alt and Honnorez, 1984; Jakobsson and Moore, 1986; Pauly et al., 2011; Stroncik and Schmincke, 2001). Palagonite is the orange or brown gel-like substance around the olivine grain in Figure 1. Alteration of glass begins on the surface of the rock or anywhere in contact with seawater such as along fractures, vesicles, or microcracks. Over time the alteration front progresses towards the interior and the entire rock becomes

altered. In Figure 1, an example of an alteration front is visible on the right hand side of the photograph between the fresh basalt glass and the orange palagonite. Figure 3 also contains examples of alteration fronts between fresh and altered glass. Prolonged water rock interaction will cause further deposition of phyllosilicates and oxyhydroxides, as well as zeolites and carbonate minerals.

Abiotic vs. biotic alteration of volcanic glass

Alteration begins at the outer edge of the glass and advances towards the interior of the rock (Figure 3A and 3B) (Furnes et al., 2001a; Furnes and Staudigel, 2006; Pauly et al., 2011; Stroncick and Schmincke, 2002). Abiotic alteration produces smooth layers that are sometimes banded because of chemical and thermal diffusive processes. The top of Figure 3A shows well defined abiotic palagonite. In contrast to abiotic alteration, biotic alteration textures (Figure 1 and Figure 3A and 3B) can be distinguished because their morphology is more complex and irregular (Fisk and McLoughlin, 2013; Furnes et al., 2001a; Furnes and Staudigel, 2006; McLoughlin et al., 2010a).

The characteristics that distinguish biotic alteration from abiotic alteration are the (1) shape and size variation; (2) biotic forms asymmetrical borders along glass surfaces such as fractures, voids, varioles, and around crystals in contrast to symmetrical or regular borders, and (3) has complex morphologies (Figure 3A, B). Palagonite is associated with abiotic and biotic alteration.

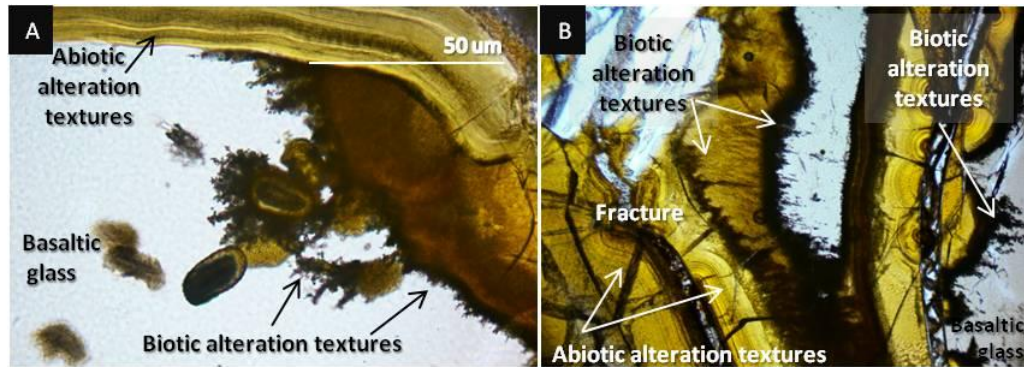


Figure 3. Photomicrograph of abiotic alteration and biotic alteration textures.

Photomicrograph of sample number 335-5-3-7 is pictured in (A) and (B). Sample is 16.5 Ma, Atlantic Ocean, FAMOUS site along the Mid-Atlantic ridge.

(A) Yellow to dark orange and brown palagonite alteration front. Banded abiotic alteration front is located in upper portion of panel (A) is a smooth boundary in contrast to the irregular, asymmetric, complex dark brown tubes protruding into the fresh basaltic glass in lower portion of panel. Note variable morphology of biotic alteration in contrast to the banded palagonite. Banded palagonite is abiotic.

(B) Photomicrograph shows multiple alteration fronts. Abiotic alteration is banded and is symmetric around fracture in the lower left corner of the panel in contrast to the irregular shape and size of the dark biotic alteration tunnels on the right side of the panel.

1.3 Previous work

The subsurface biosphere of the ocean crust has been explored for about two decades with startling discoveries such as the survival of microorganism communities in sediment up to 500 meters deep, e.g., Parkes et al., (1994). The discovery of microbes in deep sediments overlying the igneous crust has led to the suggestion that the igneous crust is a candidate for microbial life. In addition the circulation of nutrient rich fluids in the subsurface does not end at the sediment basement interface and instead veritably extends into the igneous basement (Tunnicliffe, 2003) providing a mechanism for nutrient and cell transport. The textures that I call biotic and are studied in this project (Figure 1 and Figure 3) are thought by many to be indicative of the igneous subsurface biosphere.

Investigations into the igneous subsurface biosphere are conducted to identify the characteristics, and limits, of life below the seafloor (Edwards et al., 2012; Heberling et al., 2010; Orcutt et al., 2011; Wankel et al., 2011). Determining the cause of biotic textures in volcanic glass is important for our understanding of how biological activity in this environment influences and mediates geochemical cycles (Heberling et al., 2010; Staudigel et al., 1995; Staudigel and Hart, 1982; Wankel et al., 2011; Yatabe et al., 2000). A subsurface habitat in the world's ocean basins has implications for the global cycling of metals, nutrients, and carbon in the ocean and the crust (Bach and Edwards, 2003; Edwards, et al., 2012; Furnes et al., 1999; Knowles et al., 2012; Staudigel and Hart, 1982; Wankel et al., 2011).

Textures within subseafloor volcanic glass were first documented in 1969 and were described as abiotic "hair-like" or channel features (Morgenstein, 1969). Since then, numerous observations of textures in thin sections of basaltic glass from the ocean crust have been documented. Studies have been conducted to document the location and characteristics of these textures and any evidence that is thought to demonstrate the process that creates them.

Descriptions of the textures now include two general subcategories-granular and tubular (Figure 3 and Figure 4). Granular morphology is typically described as solid

bands, semicircles, and irregular patches of spherical bodies (Figure 4) (Banerjee and Muehlenbachs, 2003; Furnes et al., 2001a; Furnes et al., 2008; Staudigel et al., 2008). Tubular textures are more complex and vary from simple smooth tubes, to curved and knotted tunnels containing small objects (Alt and Mata, 2000; Fisk and McLoughlin, 2013; McLoughlin et al., 2009, 2010).

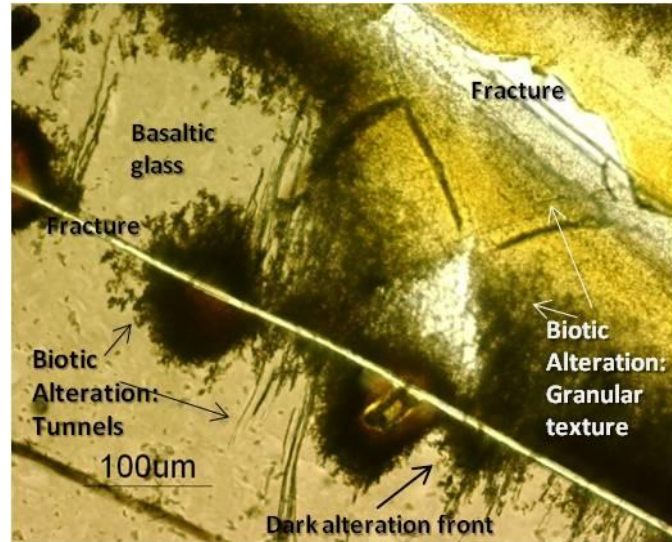


Figure 4. Photomicrograph of granular and tubular biotic alteration textures. Sample number 337-15-2-77 is 160 Ma from the Ontong Java Plateau, Pacific Ocean. Granular texture and tunnels are pictured. Fracture in upper right is the origination point of the biotic alteration. Palagonite is yellow to dark brown with a dark alteration front advancing into the basaltic glass. A fracture cross-cuts this alteration front in the lower portion of the image that possibly created the the elliptical dark textured regions at a later time then when the initial dark alteration front formation.

1.3.1 Nomenclature

Various published names for alteration textures in volcanic glass are presented in (Appendix B). The names are divided into those that indicate a biotic origin and those that are without interpretation. The list illustrates the many investigations into how the textures form and their relationship to the surrounding environment or ecosystem. The variety of the names on the list also exemplifies the controversy surrounding their potential biotic origin and how many studies have been conducted to investigate this issue of biogenicity e.g., Alt & Mata, 2000; Fisk, 1998; Fisk et al., 2013; Furnes and Staudigel, 1999; Knowles et al., 2012, 2013; McLoughlin et al., 2010a; McLoughlin, 2010b; Torsvik et al., 1998; Thorseth et al., 1992, 1995; Staudigel et al., 2008; Staudigel et al., 1995; Zhou et al., 2001.

Whether the features referred to as biotic textures in this study (Figure 1 and Figure 3) are created by biotic processes or are created by a physical or chemical process is a subject of debate. This study is conducted under the premise that they are biotic in origin. The evidence the textures are created by biotic processes includes the relative enrichment of biologically used elements such as carbon, nitrogen, phosphorus, iron, and sulfur, and organic evidence such as the presence of DNA in contrast to abiotic alteration (see Banerjee et al., 2006; McLoughlin et al., 2010, 2009 for further discussion). Evidence that secondary minerals have preserved organic carbon and nitrogen on the texture walls supports the possibility that they are biotic (Wacey et al., 2013). Both sides of the debate on biogenicity are presented in this section.

In addition, preliminary results from this study contributed to the decision to call these textures biotic. These results suggest there is a relationship between biotic alteration amount and type and the environmental conditions.

1.3.2 Evidence for biotic alteration

The subsurface biosphere in the igneous ocean crust (Figure 2) is the largest habitable environment on Earth by volume (Beth and Edwards, 2003; Orcutt et al., 2011).

Primary production biomass estimates based on what could be supported by the available energy released in water rock interactions in the ocean subsurface were made in a study by Beth and Edwards (2003). Their findings were that about 1×10^{12} g C per year is possible from a combination of oxidation or reduction of iron and sulfur, nitrate reduction, hydrogen oxidation and methanogenesis (Bach and Edwards, 2003). These estimates suggest that water-rock interactions rival deep sediment heterotrophic systems, and illustrate the importance of subsurface basaltic ecosystems on a global scale.

Morphology

Although the morphology is not the strongest evidence, the variety of branching shapes and complex morphology (Figure 3), have caused some authors to speculate and even conclude the textures are biotic in origin (Furnes et al., 2002; McLoughlin et al., 2009, 2010; Staudigel et al., 2008; Torsvik et al., 1998; Thorseth et al., 1992). Further, the textures along the glass alteration front have a “biotic” morphology in contrast to the smooth alteration front that might be expected for a chemical reaction boundary (Figure 3) (Fisk, 1998; Furnes et al., 2007, 2002; Furnes and Staudigel, 1999; Knowles et al., 2012).

The other morphological characteristic that has led some authors to suggest of a biotic origin is that textures are primarily found anywhere glass is accessible to seawater and alteration has occurred (Figure 1 and Figure 3) (Banerjee et al., 2005; Fisk and McLoughlin, 2013; Fisk et al., 1998; Furnes et al., 2001a ; Furnes et al., 2008; Furnes and Staudigel, 1999; Heberling et al., 2010; Josef, 2006; Knowles et al., 2012, Knowles et al., 2013; McLoughlin et al., 2009; Morgenstein, 1969; Santelli et al., 2008; Staudigel et al., 2008, Staudigel et al., 2009; Staudigel et al., 1982; Storrie-Lombardi and Fisk, 2004; Thorseth et al., 2002, Thorseth et al., 2003; Walton 2007, 2008). This includes observations of textures within and along alteration fronts, along fractures, outer pillow margins, vesicle walls, glass shard boundaries, and other open space.

The biotic textures develop asymmetrically on opposite sides of fractures, and have varying degrees of smoothness (Figure 3) (Furnes et al., 2007, 2002; Furnes and

Staudigel, 1999; Staudigel et al., 2008; Storrie-Lombardi and Fisk, 2004). In addition, bacteria resembling the shape and size of mineralized, oval-shaped bodies have been observed on altered glass containing the textures (Alt and Mata, 2000; Furnes et al., 2002; Thorseth et al., 1992, 2003).

Inorganic chemical evidence

The observed oxidation state of iron and manganese in basalt is attributed to microbial reduction or oxidation processes that are related to the alteration of basaltic glass. For example, chemical evidence of biotic activity includes iron oxidation products associated with the textures in contrast to fresh glass or abiotic alteration (Bach & Edwards 2003; Templeton et al., 2005). Other potential biological signatures found include depleted magnesium (Mg^{2+}), iron (Fe^{3+}), calcium (Ca^{2+}) within the biotically altered glass e.g., Alt and Mata, 2000; Furnes et al., 2001a; Knowles et al., 2013; Staudigel et al., 2008 Thorseth et al., 1992. These three elements are all essential to life. Mapping the Fe^{3+} and manganese (Mn^{2+}), and Ca^{2+} across the glass and altered glass region has consistently revealed oxidized Fe^{3+} in the fracture fill, and depleted levels of Fe^{2+} , Mn^{2+} , and Ca^{2+} in proposed biotic textures compared with the levels in fresh glass (Alt and Mata, 2000; Knowles et al., 2013; Staudigel et al., 2008).

Levels of nitrogen and phosphorus that are abundant in many organic compounds are higher in the biotic textures than in the abiotic textures (Alt and Mata, 2000; Giovannoni et al. 1996; Torsvik et al. 1998; Furnes & Muehlenbachs 2003; Staudigel et al., 2008). Stable isotopes commonly indicative of biotic activity such as depleted $\delta^{13}C$, relative to fresh glass has also been observed (Torsvik et al., 1998). Within tunnels in meta-volcanic Archean pillow basalts carbon that is interpreted to be biotically introduced has been found (Banerjee et al., 2007).

Organic chemical evidence

Inside the biotic alteration tubes traces of organic molecules in the form of aliphatic hydrocarbons, amides, and carboxylic acids, all components of lipids, proteins, and

carboxylic acids have been identified using infrared spectroscopy (Preston et al., 2011). The search for organic material or nucleic acids within the tunnels has revealed DNA at the fresh glass-altered glass interface (Alt and Mata, 2000; Giovannoni et al. 1996; Torsvik et al. 1998; Banerjee & Muehlenbachs 2003; Walton & Schiffman 2003). Using DNA-specific staining, a study conducted by Thorseth et al., (1995b) found DNA containing cells that are the same size as microbes. However, this could suggest contamination because it is difficult to know whether the DNA within the tunnels, was deposited before, during, or after the tunnels were created (Knowles et al., 2013; Furnes et al., 2001a; Staudigel et al., 1995; Thorseth et al., 1995b).

Carbonaceous linings preserved by secondary minerals within Troodos Ophiolite volcanic rocks (~92 Ma), were found using focused ion beam milling combined with transmission electron microscopy, and scanning electron microscopy (Wacey et al., 2013). The use of these three techniques enabled nanoscale organic carbon filaments and sheets and nitrogen within and contouring the tunnels to be detected in the volcanic glass.

Microbial colonization

When microbes colonize a glass surface in a laboratory, the most commonly produced textures are pits (Thorseth et al., 1995b). These pits are caused by a local reduction in pH due to organic acids created through biotic activity. In another study, microbial colonization of a glass surface increased the dissolution of volcanic glass when compared to abiotic corrosion (Furnes et al., 2001a; Knowles et al., 2012; McLoughlin et al., 2010b; Santelli et al., 2008; Thorseth et al., 1995). Staudigel et al. (1995) observed the dissolution of glass after applying a microbial system to the surface of volcanic glass. So far though, no laboratory studies have produced complex biotic textures in glass such as those illustrated in Figure 1, Figure 3, and Figure 4.

Experimental studies have shown the formation of a biofilm that is enriched in iron on glass with microbes in contrast to glass without microbes under the same laboratory conditions (Aouad et al., 2006).

1.3.3 Evidence for abiotic alteration

Several proposals for abiotic processes are listed below. Most abiotic textures listed below have been replicated in a laboratory; however, the diversity of naturally occurring tunnels called biotic here has not been replicated to date.

Alpha recoil track decay and fission tracks

Alpha recoil track decay (ART) from the radioactive decay of uranium and thorium in basaltic glass, mediated by pressure solution alteration, produces biotic like textures (French and Blake, 2012). Numerically modeled radioactive decay demonstrated that textures from fission tracks and alpha decay can be produced, and also will increase in abundance with age. Fission tracks are larger than the biotic textures discussed here however, and ART are smaller than the biotic textures in glass.

Hydrofluoric acid etching

Perhaps the most compelling evidence for abiotic texture formation is supported by an experiment in which 1% hydrofluoric acid (HF) was applied to basaltic glass as an analog for more slowly acting organic acids (Fisk et al., 2013). The HF with a pH of 2.8 was used to simulate hydrothermal conditions. The HF created circular pits, called “etches”, and penetrating tunnels ~5 microns in diameter. These tunnels, especially those running perpendicular to the glass surface, resemble one type of biotic texture. The comparison of natural textures with those produced in the lab with HF is important because it suggests that acidic conditions produce the textures and microbes use the structures created for habitat after dissolution.

Tensile fractures

A feature similar to the biotic textures that occurs in unaltered vesicle walls is presented here because they are tubular features (French and Muelenbachs, 2009). However, they are nanometer sized in contrast to the micron sized textures studied in this thesis. They are tangential tensile fractures produced when a layer of magmatic fluid is

separated from a vesicle interior by hot glass. As vapor pressure in the vesicle decreases the magmatic fluid moves towards the vesicle interior. The once hydrated glass becomes dehydrated and this density disparity creates pull-apart structures in the glass that resemble the complex morphology of the tunnels.

Seawater corrosion

Crovisier et al. (1987) conducted synthetic glass experiments and demonstrated that minor prefracture microcracks began to form after the glass interacted with artificial seawater. Because they were small with respect to the textures observed in nature that are discussed here, the conclusion was that texture formation takes a long time, perhaps too long for laboratory experiments attempting to replicate the biotic textures (Crovisier et al., 1987). This method has not been fully evaluated; longer duration experiments are required.

Abiotic methods of altered glass have not produced the complexity, variety, and size range of textures considered biotic in this study. The textures created in a laboratory setting that are the most similar to the textures studied here resemble granular texture. However, the complex morphology of what I call biotic textures in this study has not been reproduced in a laboratory.

1.3.4 Biotic texture characteristics and occurrence

Geologic province

Biotic textures are found in a wide range of geologic provinces (Appendix C) (Banerjee and Muehlenbachs, 2003; Edwards et al., 2012; Fisk and McLoughlin, 2013; Josef, 2006; Knowles et al., 2012; McLoughlin et al., 2010b). They have been recognized in all the world's major ocean basins and in diverse provinces such as seamounts, ridge-flanks, ocean island basalts, continental margins, and submarine flood basalts (Cousins et al., 2009; Edwards et al., 2012; Fliegel et al., 2010; Furnes et al., 2005b; Furnes and

Staudigel, 1999; Staudigel et al., 2006; Ivarsson et al., 2012; Montague et al., 2010; Santelli et al., 2008; Schrenck et al., 2010; Walton 2007, 2008).

Host rock

Examples of biotic textures, primarily observed in basaltic rocks, are illustrated in numerous publications (Figure 1, Figure 2, and Figure 3) (Banerjee and Muehlenbachs, 2003; Edwards et al., 2012; Fisk and McLoughlin, 2013; Knowles et al., 2012; McLoughlin et al., 2010b), although there are some exceptions. For example, within a subglacial basalt deposit in Valafell, Southern Iceland “pits” and tubular features were reported within obsidian and rhyolite samples from the same location (Cockell, et al., 2009; Herrera et al., 2008). Ontong Java, the world’s largest volcanic plateau is host to a volcanoclastic tuff with granular and tubular textures (Banerjee and Muehlenbachs, 2003). Although several instances of terrestrial and terrestrial-marine transition examples have been documented, including biotic textures found within a subglacial deposit in Antarctica, examples from subseafloor volcanic glass are the most common to date (Cousins et al., 2009; Fisk et al., 1998, Fisk et al., 2006; Fisk and McLoughlin, 2013; Knowles et al., 2012; McKinley and Stevens, 2000).

Host material containing very similar tunnel texture includes carbonate brachiopod shells (Buijs, et al., 2004). The carbonate material containing thousands of microborings could be a carbon source in addition to providing protection. In a study conducted by Cherchi et al., 2012, microborings were found in foram shells.

Evidence of microbial activity in a terrestrial environment was found in the Rattlesnake Tuff from the Blue Mountain Region of Oregon (Fisk et al., 1998). A biofilm containing colonies of microbes was found coating glass shards on a sample that was subject to dessication, and temperature variations of about 50°C.

Observations of biotic textures in continental flood basalts have not been made yet even though such systems are reported to host microbes (Stevens and McKinley, 1995),

and basaltic rock is a viable substrate for colonization (McLoughlin, 2009; Thorseth et al., 1995).

Depth

Basaltic glass containing biotic textures is found on seafloor outcrops and at a range of depths below the seafloor (Appendix C). The maximum basement, or basalt depths, recorded to date for biotic textures coincides with the known depth limit of fresh glass, approximately 550 meters or halfway through the basaltic layer (Figure 2) (Furnes et al., 2002). However, drilling has occurred primarily in the shallowest reaches and samples from great depths are limited.

Age

Because oceanic lithosphere is destroyed at subduction zones the oldest in-place oceanic crust is ~170 Ma, therefore this is the age of the oldest *in situ* samples of biotic textures (Fisk et al., 1999; Furnes et al., 2002). The youngest rocks that include examples of biotic tunnel alterations are from the quaternary (Furnes et al., 2002; Banerjee et al., 2006). While these are the ages of the host rocks, they may not be the age of the biotic textures. This study seeks to investigate the relationship between age of the crust and the alteration textures.

Abundance

In order to gauge how much of the ocean crust is effected by biotic alteration; the quantity of rock effected by biotic alteration has been estimated and several environmental controls on abundance have been observed including depth and salinity. Furnes and Staudigel (1999) and Furnes et al. (2001a) estimated the percent of biotic alteration of subseafloor basaltic glass and found that abundances ranged from <10% up to 90%. Cousins et al., (2009) corroborated this wide range of possible abundance values during an investigation of sub-glacial basaltic glass from a marine-glacial melt water transition environment in Antarctica. The rocks contained a range of 20% to 100% biotic alteration. The salinity in the water was correlated with increasing abundance. The rocks

from the dominantly marine environment had the highest amount of biotic alteration, ranging from 60% to 100% biotic. Another environmental control of biotic alteration abundance is depth. In the upper 250 meters of the basaltic layer biotic alteration is most abundant and then it decreases with depth (Furnes and Staudigel, 1999; Furnes et al., 2001a).

Biotic texture size

Biotic textures are typically micron sized (Alt and Shanks, 2011; Banerjee and Muehlenbachs, 2003). Tube or tunnel dimensions vary from less than a micron long up to >200 microns long (Figure 1, Figure 3, Figure 4) (Fisk and McLoughlin, 2013; McLoughlin et al., 2009; Walton, 2008). Documented width variation ranges from ~1 to 10 microns (Alt and Mata, 2000; Banerjee et al., 2006). Granular dimensions vary from ~0.5 < up to ~3 microns and are generally equidimensional (Alt and Mata, 2000; Banerjee and Muehlenbachs, 2003).

1.3.5 Modern biotic textures and ancient life

Evidence generally accepted for early life on Earth includes outcrop scale structures, fossilized microbial mats, microscopic fossil textures, and isotope fractionation (Banerjee et al., 2006). Biotic textures similar to this study have been observed in rocks from the Archean to the Quaternary and are interpreted to be microscopic fossil texture evidence of early life on Earth (Banerjee et al., 2006; Fliegel et al., 2010; Furnes et al., 2002; Furnes et al., 2004). The oldest known examples of these textures analogous to modern examples are mineralized granular and tubular textures in ~3.4-3.5 Ga metamorphosed ophiolites from Barberton, South Africa (Banerjee et al., 2006). Granular and tubular textures are also found preserved by titanite mineralization in pillow lavas in the Pilbara Craton, Western Australia (3.35 Ga). These examples of biotic textures in rocks from an ancient volcanic seafloor are interpreted by some to be the oldest evidence of life on Earth (Banerjee et al., 2006). Because the host rocks are pillow lavas and interpillow hyaloclastite remnants, these ancient textures have a compelling relationship to modern

textures, and therefore are compelling evidence of early life on Earth (Banerjee et al., 2006).

The gap in information between the Barberton example and the modern ocean has led to examination of ancient rocks, such as the Jormua Ophiolite Complex, Finland from ~1.95 Ga, which shows evidence of biotic activity in the form of carbon isotope signatures (Furnes et al., 2005a). These isotopes are interpreted as relics of biotic activity even though no biotic textures are present (Furnes et al., 2005a). The Wutai Group contains textures in meta-volcanic pillow lavas (~1.8 Ga) from the Greenstone Terrane in the North China craton (McLoughlin et al., 2010a).

Establishing a biogenic origin for these ancient features would be supported by finding modern analogs. Thus, understanding the environmental conditions in which the textures are found today in volcanic glass can increase our confidence of a biotic interpretation of the Archean textures and could provide insight into the ecosystem present during the proliferation of early life.

1.4 Statement of the Problem

To resolve whether the textures are biotic requires understanding the parameters of subseafloor basaltic ecosystem. While this study cannot resolve this large scale question, it is a step towards understanding the physical and chemical characteristics of the environment. The principle that the present is the key to the past is commonly applied to the marine sedimentary record, especially in terms of biotic structures. For example, material such as the skeletal remains of calcareous algae, or other crystalline material such as ooids, or fine sediment, that is created through microbial processes leaves a physical record of the formation conditions (Goffredo and Dubinsky, 2013). The composition, mineralogy, and age all serve as evidence of the habitat conditions. Many of these calcareous and siliceous marine organisms have modern analogs that have been used to verify the habitat conditions evident in the rock record. The presence of these fossils can then be used by themselves to infer, temperature, pH, fluid and substrate chemistries once a connection between habitat and species is made. Because this

comparison arose out of abundance and distribution correlations with environmental characteristics and was sometimes verified with modern analogs, I assert that it is a method that can be applied to biotic textures in basaltic glass.

There are two main objectives of this study. The first is to investigate how biotic alteration varies depending on conditions in the environment as evidenced in the rocks. The second is to determine if these variations can be useful for defining an ecosystem. The information garnered from these correlations has the potential to be used as a starting point for future investigations into viable ecosystem conditions. The first objective will be accomplished by describing and quantifying the correlation between the texture morphologies and relative abundance, and the environmental conditions. The second objective will be accomplished by interpreting the environmental conditions in terms of fluid chemistry, temperature and pH.

My hypothesis is that the morphology and abundance of textures within altered subseafloor volcanic glass varies with the environment. Conditions to be investigated include sample age and depth into basalt, overlying sediment thickness, *in situ* temperatures, and conditions inferred from secondary mineral proxies.

Many examples of textures in basaltic glass observed in the modern ocean crust are used here to investigate this hypothesis. DSDP and ODP samples and associated environmental data from the modern ocean provide an opportunity to address the objectives of this study in detail. Information collected with these samples, includes, sample age and depth into basalt, overlying sediment thickness, secondary mineralogy, as well as information about the geologic province of the rocks. This information provides a basis for this investigation.

This study is conducted with the premise that biotic activity creates these textures. The premise that these textures are biotic means there were ecosystem conditions driving the occurrence and distribution of the species creating the textures. These ecosystem drivers could be evident in the rocks containing the textures. Subseafloor basaltic glass is within a viable ecosystem with the ingredients necessary for life including, appropriate

temperature ranges, adequate nutrients, and fluids (Munn, 2003). This research has the potential to aid future studies about the relationship between these textures and the subseafloor biosphere. If the premise that they are biotic is demonstrated in future studies to be false, understanding of the environmental conditions still provides illumination of the mechanism creating the textures, or at least narrows the range of possibilities.

In this study, the term *biotic alteration* describes the physical evidence of how a sample has been changed due to presumed biotic activity. This term is defined in the same way as studies by Staudigel et al. (2008), and Furnes et al. (2008). Biotic alteration is described in this study by two components. The first component is *biotic alteration abundance*. This component is defined as the percentage of alteration deemed to be biotic relative to the total alteration along glass surfaces in the samples. The second component is *biotic alteration texture type*. This component is defined by morphology and size using a previously created classification scheme for textures in glass alteration (Fisk and McLoughlin, 2013). These two components provide a way for biotic alteration to be compared to the environment.

The environmental parameters chosen are sample age (I), sample depth into basalt (II), overlying sediment thickness (III), temperature (IV), and secondary mineralogy (V). Previous studies have suggested the importance of the relationship between biotic alteration and age, temperature, depth, and secondary mineralogy because they provide context for the alteration genesis and serve as proxies for other conditions (Furnes et al., 2001a, 2001b; Furnes and Staudigel, 1999). These parameters are described in the methods, section 2.3.4.

2. METHODS

My hypothesis is that biotic alteration in basaltic glass varies with the environment conditions associated with the glass (Appendix C and Appendix F); testing this hypothesis involved two stages of data collection. The first is to estimate biotic alteration abundance and determine the presence of specific biotic textures. During the second stage of data collection environmental information was gathered. This included previously compiled data: sample depth into basalt, sample age, and sediment thickness overlying the samples (Appendix C) (Josef, 2006). Secondary mineral observations were also obtained from published data collected with the samples (Appendix F). A survey for the presence of sulfide minerals in the samples, and modeling of the *in situ* temperature conditions was also conducted (Appendix D). These two types of data, environmental and biotic alteration, were then investigated.

2.1 Sample source

This study used eighty samples collected by the Deep Sea Drilling Project (DSDP) and the Ocean Drilling Program (ODP) (Figure 5). The DSDP and the ODP were selected as a sample source because of the large global collection of seafloor basaltic glass samples available with supplemental environmental information about the samples and locations. These samples were made into thin sections by the DSDP and ODP and also from subsequent subsampling of the cores (pers. communication, M.Fisk).

DSDP (1966-1983) was the first of three collaborative international drilling programs. Their primary objectives included drilling to investigate the oceanography, geology, and geophysics of the world's ocean basins (Nierenberg, 2007; ODP, 2007). In total 624 sites were cored during DSDP operations providing many advances in marine geosciences and plate tectonics. The maximum depth drilled into the seafloor was 1740 meters; 7044 meters was the deepest water drilled operations were conducted in.

The ODP (1983-2003) was the successor to the DSDP (ODP, 2007). Their primary objective was to conduct ocean coring and downhole logging to explore Earth's history

and the structure of Earth's ocean basins. A total of ~ 1800 sites were explored in the Pacific, Atlantic, Southern, Arctic, and Indian oceans during ODP operations. ODP drilling achieved depths of approximately 600 meters and ranged from between 50 and 400 meters (ODP, 2007). This study strives to align with the objectives of the DSDP and the ODP to conduct both ocean and geological exploration.

Thin sections of samples were selected from a collection of over a hundred samples from studies conducted by Josef (2006), and Fisk and McLoughlin (2013). The sample criteria for this study were aligned with the studies by Josef (2006) and Fisk and McLoughlin (2013). These criteria were tailored to fit this study, and eighty samples fit all of the criteria, listed below.

These criteria include (1), all thin sections contain basaltic glass and (2), secondary mineralization and alteration of the glass had not occurred to the extent that texture morphology was unidentifiable. (3) The environmental parameters selected for this study were available, including sample depth into basalt, sample age, sediment thickness overlying the sample site, and secondary mineralogy observations. Lastly (4), a wide range of geographic and tectonic environments and variable ages and crustal depths were used.

The samples were selected from a diverse range of environments because the exact mechanism creating the textures remains unknown. To maximize the diversity of the samples in this study only one or several samples per location were used to conduct these analyses. This allowed the use of many locations in contrast to many samples from a few locations to insure the samples are representative of a global population. The samples cover much of the globe including the Atlantic, Indian, Mediterranean Sea, and the Pacific Ocean basins (Appendix C and Figure 5). Geographic diversity includes Gulf of California, Mid-Atlantic Ridge, Barbados Ridge Complex, Argo Abyssal Plain, Chilean Triple Junction, Emperor Seamounts and many others. Geologic provinces represented are large igneous provinces, hot-spot tracks, ocean ridge flanks, back arc basins, and others.

Overlying sediment depth at the sample drill sites, ranged from 22.1 meters (Hole 862C) to ~1404 meters (Hole 793), sample ages range from 0.5 Ma (Hole 482D) to 156 Ma (Hole 105), and basalt drilling depths from the seafloor range from ~0.3 meters (Hole 266) to 537.8 meters (Hole 418A) (Josef, 2006) (Appendix C).

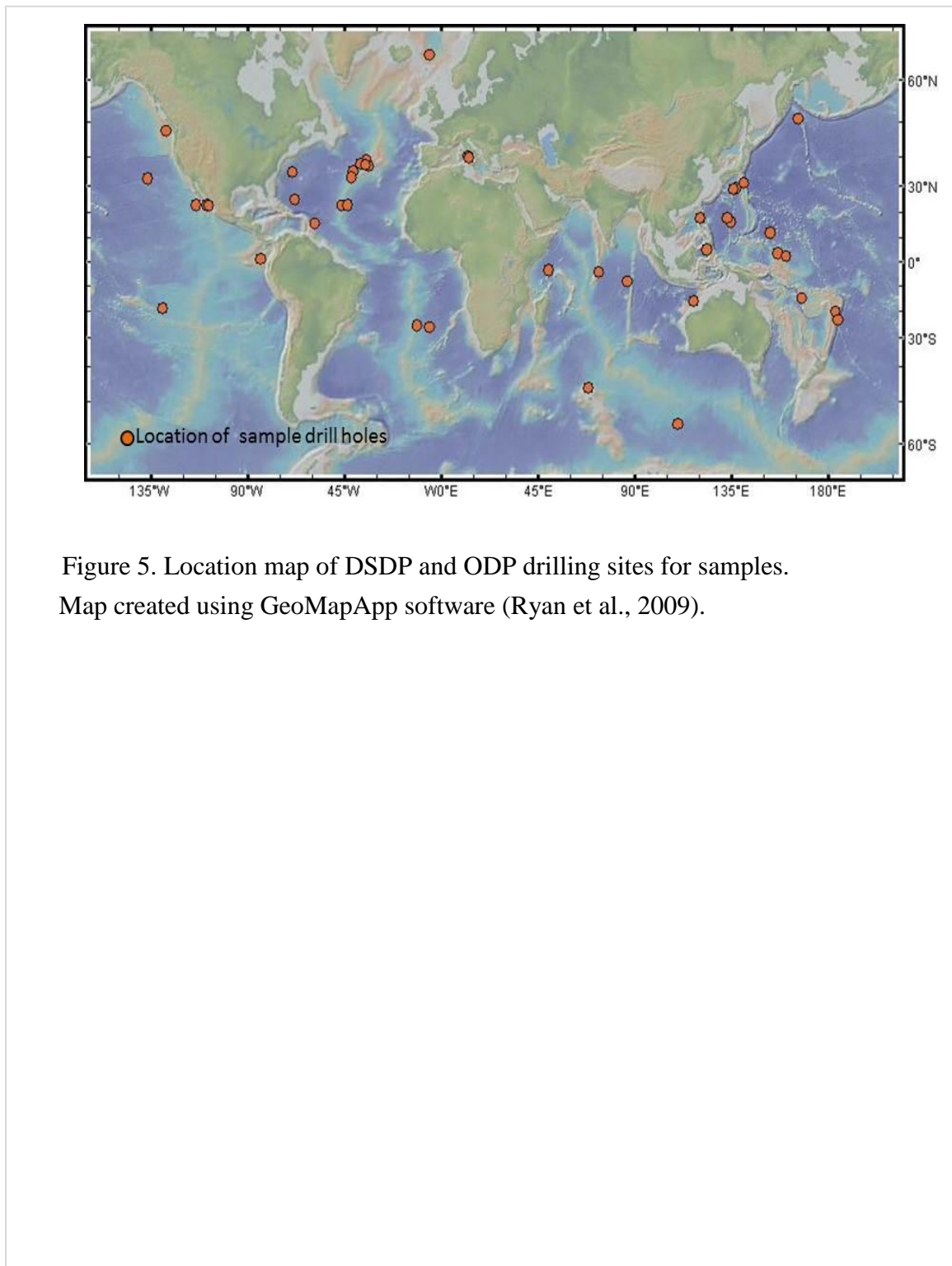


Figure 5. Location map of DSDP and ODP drilling sites for samples. Map created using GeoMapApp software (Ryan et al., 2009).

2.2 Data description

The components of biotic alteration, abundance and texture type, within thin sections of basaltic glass samples were documented using a transmitted and reflected light microscope (Olympus BX60 BMAX), equipped with a digital camera (Nikon DXM200). The microscope was also equipped with 2.5x, 10x, 20x, and 50x objective lenses.

2.2.1 Biotic alteration abundance

Biotic alteration abundance is used to numerically evaluate the percent of rock that contains evidence of biotic activity in terms of all the alteration present in the rock (Figure 6). The abundance of biotic alteration was estimated in increments of 10% as a percentage of the total alteration along glass margins with a method adapted from Cousins et al., (2009) (Figure 6). Because biotic alteration occurs wherever water comes into contact with glass, it originates along the outer surface of the rock, the edges of mineral grains and vesicles (Figure 1), fractures (Figure 4) and varioles (Figure 6) and any other void space (Cousins et al., 2009; Fisk et al., 1998; Fisk and McLoughlin, 2013; Furnes et al., 2001a; Furnes and Staudigel, 1999; Staudigel et al., 1995; Giovanonni et al., 1996; McLoughlin et al., 2010; Montague et al., 2010; Thorseth et al., 1992).

The locations of alteration in the thin sections were identified using the 2.5x objective lens and confirmed using the 10x objective lens. The areas were surveyed with 10x and 20x lenses to distinguish the biotic alteration (Figure 3 and Figure 6) from the abiotic alteration. The locations were confirmed by alternating between the 10x, 20x, and 50x objective lens (Table 1). Once the difference between the biotic and abiotic alteration was verified, the abundance estimate for the sample was made by averaging between five and ten linear features (Figure 6). A final confirmation of the estimate was made by alternating between the 10x, 20x, and the 50x objective lenses to ensure that it was representative for the whole sample (Figure 6).

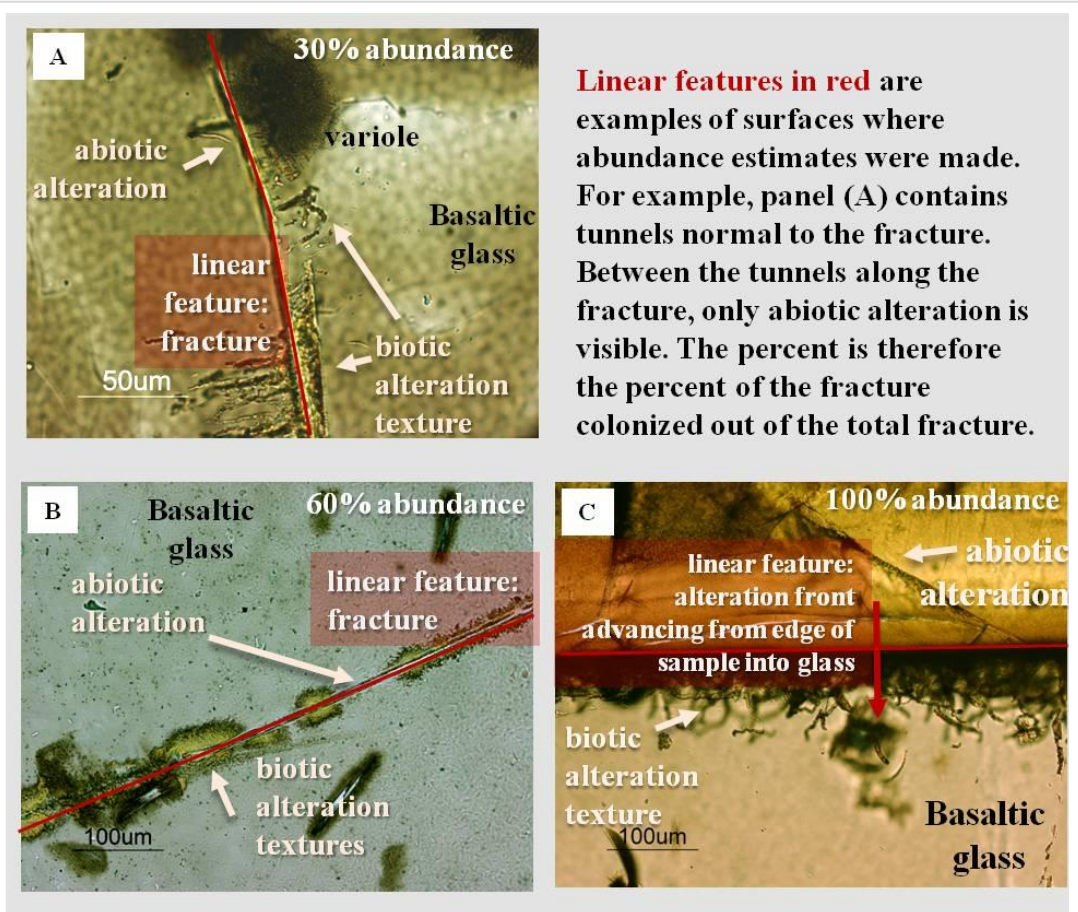


Figure 6. Photomicrographs of biotic alteration used for abundance estimation.

A. Example of 30% biotic alteration. Linear feature is a fracture with palagonite. Biotic textures are empty thin tunnels. Sample number 442B-19-1-12 is 18 Ma, from the Shikoku Basin, Pacific Ocean.

B. Example of 60% biotic alteration. Linear feature is a fracture. Biotic texture is mossy, yellow palagonite. Sample number 240-7-1 is 53 Ma and is from the Somali Basin, Indian Ocean.

C. Example of 100% biotic alteration. Linear feature is alteration front advancing into the glass away from the sample edge. Sample number 105-41-3-45 is 156 Ma from the lower continental rise hills, Atlantic Ocean.

2.2.2 Biotic alteration texture identification

Biotic texture classification (Figure 7 and Figure 8 and Appendix A) provides a way to compare the morphological evidence of biotic activity with the environment. The objective of the survey of these textures was to classify them based on morphology using a system created by Fisk and Mcloughlin (2013) (Figure 7 and Figure 8). The classification system contains 28 texture types, divided into 26 tunnel types, and two others: buds and bubbles, and granular texture (Figure 7, Appendix A). The samples were surveyed for the presence and absence of the 28 texture types (Table 1).

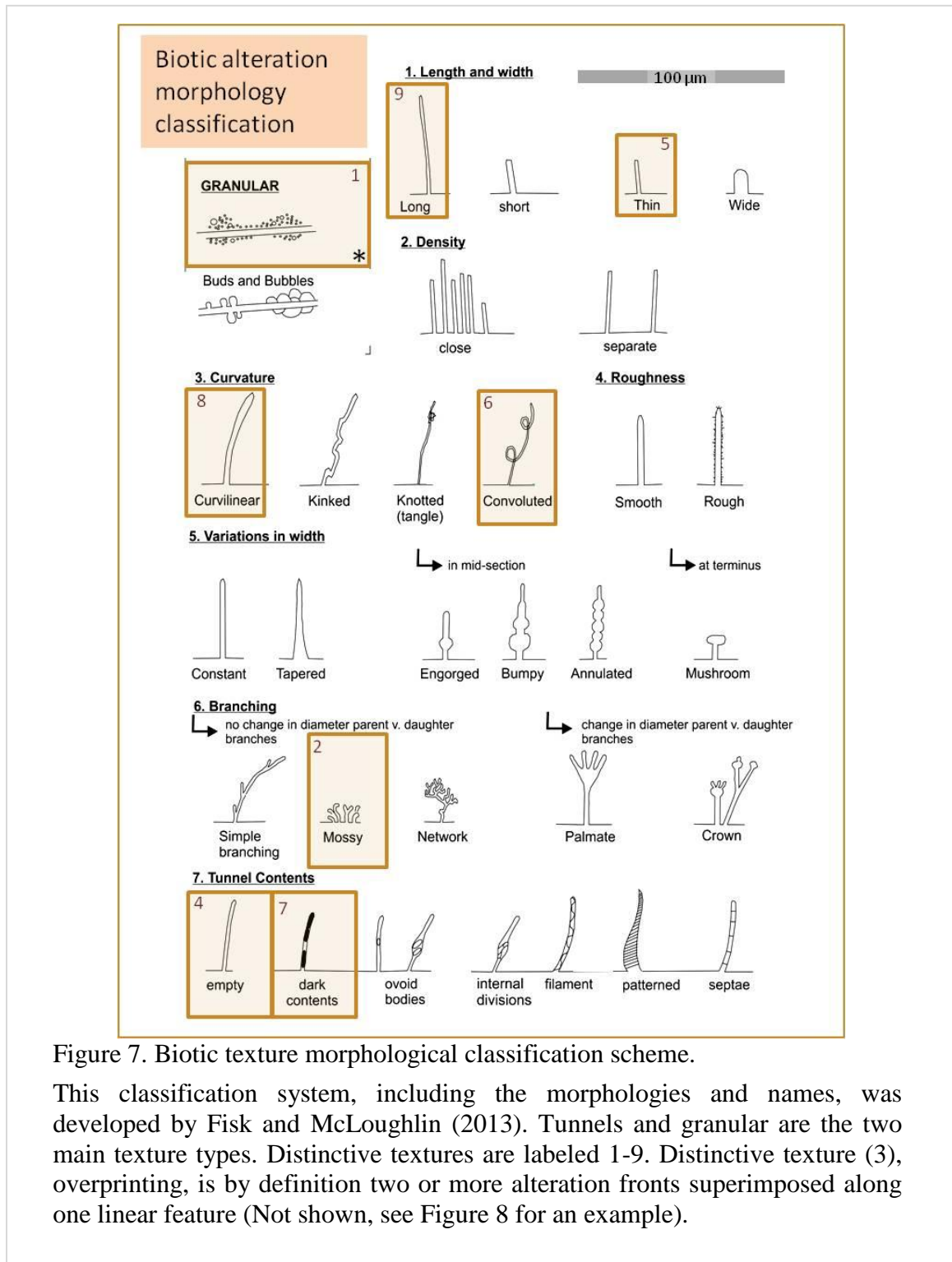


Figure 7. Biotic texture morphological classification scheme.

This classification system, including the morphologies and names, was developed by Fisk and McLoughlin (2013). Tunnels and granular are the two main texture types. Distinctive textures are labeled 1-9. Distinctive texture (3), overprinting, is by definition two or more alteration fronts superimposed along one linear feature (Not shown, see Figure 8 for an example).

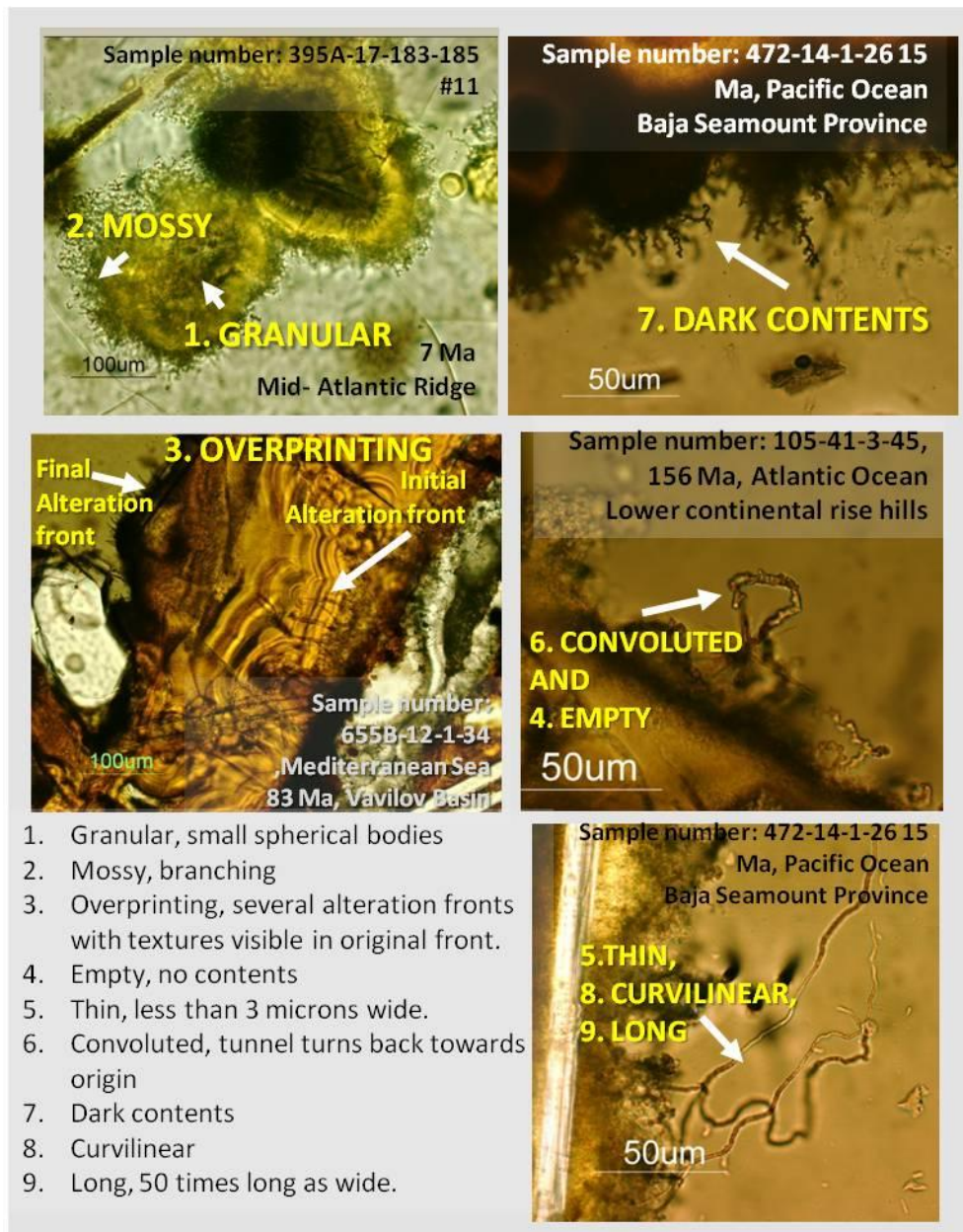


Figure 8. Photomicrographs of the nine distinctive textures in basaltic glass.

The distinctive textures are listed in yellow. Lower gray panel gives details about their morphology. Samples are from the Mediterranean, Atlantic, and Pacific Oceans. All contain light yellow to dark brown palagonite and fresh glass. More than one texture type can occur in a sample and some textures can be classified as multiple types. For example, both thin and long.

Table 1. Method used to identify biotic textures.

<i>Step</i>	<i>Magnification</i>	<i>Purpose of step</i>
Step 1	10x	Locate glass alteration boundary with biotic textures.
Step 2	20x	Survey alteration textures in the field of view located in step 1.
Step 3	50x	Identification of the texture types from step 1.
Step 4	10x-50x	*Repeated until entire thin section has been surveyed and the biotic textures present in the sample were recorded. A final survey of the thin section was conducted to ensure no texture types were overlooked or misidentified.

*Sometimes several iterations were necessary because certain texture types occurred in one location of a thin section and not in another. For example, thin tunnels might be visible in one field of view but not another.

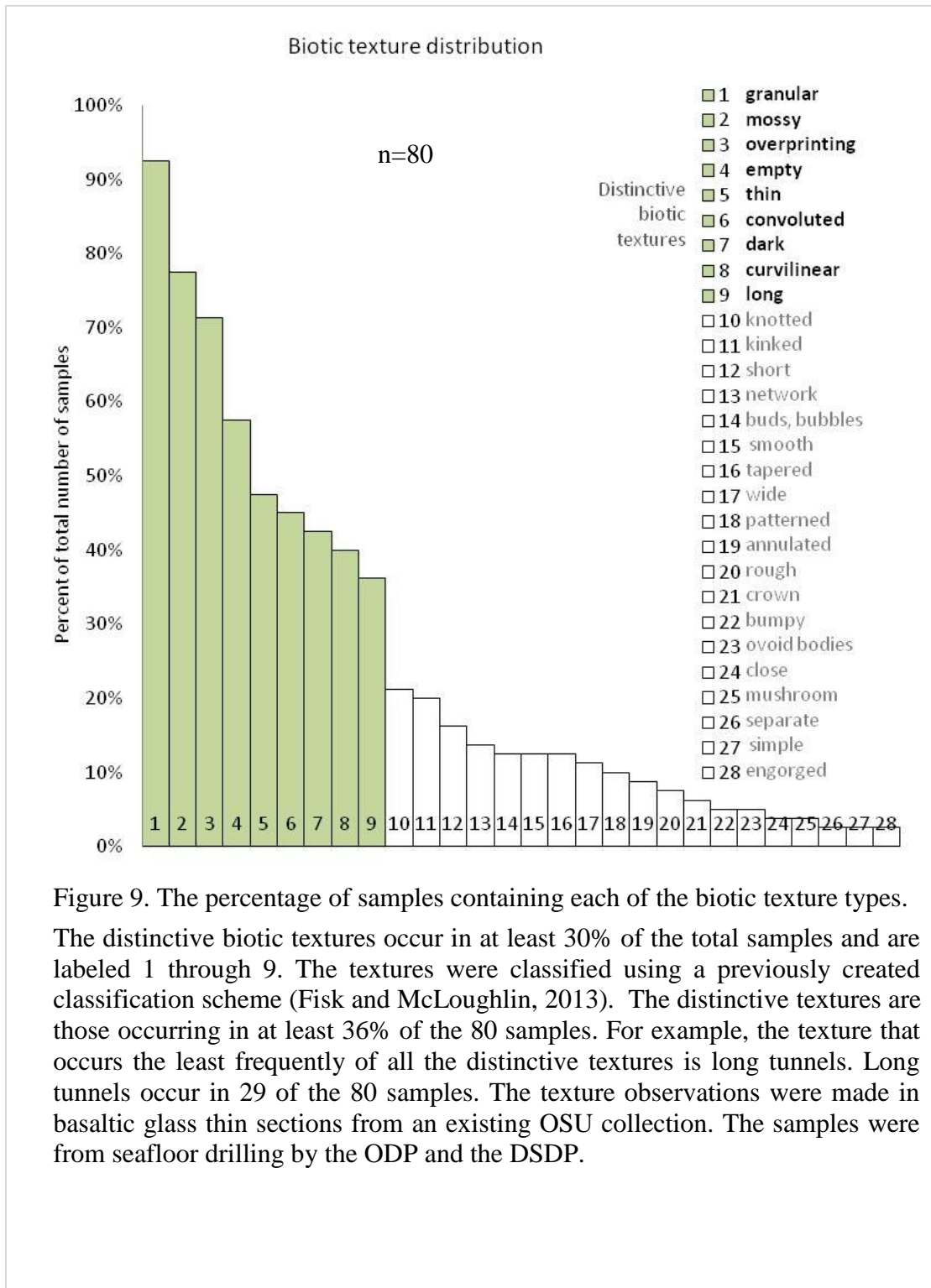
Verification of classification followed the same four-step procedure. In total the thin sections were observed thoroughly between three and four times over the course of the study, with random checks between surveys as well.

The textures occur in three dimensions causing the need for several iterations with different magnifications to accurately identify the textures (Figure 1).

2.2.3 Distinctive biotic texture identification

The distinctive textures (Figure 8) selected were present in a high number of samples to maximize the amount of samples available for comparison with the environmental parameters. Additionally, a larger sample size increases the likelihood that the correlations captured are representative of the total population.

The percentage of the 80 samples containing the respective texture types is shown in Figure 9. A division occurs at the transition from long to knotted tunnels: long tunnels occur in 36% of the 80 samples but only 21% contain knotted (Figure 9). This break in sample quantity from long tunnels to knotted tunnels resulted in each distinctive texture sample set containing over 20 samples. These are: (1) granular, (2) mossy, (3) overprinting, (4) empty, (5) thin, (6) convoluted, (7) dark contents, (8) curvilinear, and (9) long. Confirmation was also made to ensure that each of the nine types were morphologically distinct from one another, readily identifiable in thin section, and not likely to be confused with other texture types.



2.3.4 Environmental parameter selection

The environmental characteristics sample age, depth and overlying sediment thickness, were three of the five selected for comparison (Table 2). These data were obtained from the drilling program data collected at the time the cores were gathered. Josef (2006), in her master's thesis compiled this information from the ODP and the DSDP volumes for most of these samples and her compilation was used here (Appendix C). Temperature modeling (Appendix D) and secondary mineralogy from the ocean drilling program volumes (Appendix E) were used.

Table 2. Environmental parameter definitions and explanation of data collection

<i>Environmental parameters</i>	<i>Explanation</i>	<i>Data collection</i>
I. Age (Ma)	Age of the rock containing the biotic textures.	*Age dates measured by the DSDP and ODP
II. Sample depth into basalt (m)	Depth from the bottom of the sediment layer down into the subsurface to the drill core piece location.	*Sample depth into basalt from DSDP and ODP
III. Overlying sediment thickness (m)	Depth of sediment layer overlying the site where the drill core was obtained.	*Sediment depths from DSDP and ODP
IV. Temperature (°C)	Modeled ambient temperature for sediment-basalt interface	Temperature modeled as a function of age and sediment thickness (Appendix D)
V. Secondary minerals smectite chlorite carbonate(calcite) zeolites pyrite (hand sample) oxidized minerals (celadonite, iddingsite, limonite, oxyhydroxides)	Secondary alteration mineral assemblage deposited as fracture and void space fill. Oxidized minerals are grouped to represent oxidizing conditions.	Alteration mineral observations from DSDP and ODP volumes.
V. cont'd: Sulfide minerals	Secondary sulfide minerals associated with the altered regions of thin sections	Thin section observations

DSDP and ODP data records contained within initial reports and volumes were used extensively (Appendix C and Appendix E).

*Data originally from the ODP and the DSDP volumes was compiled by Josef (2006) prior to use in this study.

Environmental parameter I. Sample age

Sample age, is used in this study as a proxy for fluid and thermal conditions (Table 2) (Edwards et al., 2012; Stein and Stein, 1994) and can be used to infer the presence of oxidizing or reducing conditions resulting from crustal sealing (Furnes et al., 2001a; Jakobson and Moore, 1986). These two processes, hydrothermal activity and the resulting chemical gradients, influence the type of nutrients available for microbial metabolism (Bach and Edwards, 2003b; Edwards et al., 2012; Orcutt et al., 2011). Additionally, in a study to investigate the relationship between sample age and biotic alteration, Furnes et al., (2001a) observed equivalent amounts of alteration for three age groups, 0-6 Ma, 10 to 32 Ma, and 110 Ma, which led them to suggest three competing scenarios: that perhaps all alteration occurs early, or that the proportion of biotic alteration remains the same, or that biotic alteration can occur over a long time period. By using sample age in this study these three ideas can be investigated further.

Environmental parameter II. Sample depth into basalt

Sample depth into the basaltic layer is useful for inferring the relationship between the textures, temperature, and fluid circulation (Stein and Stein, 1994; Fisher, 1998; Fisher and Becker, 2000; Becker and Fisher, 2000). Fluid circulation is necessary for nutrient and oxidant availability. Additionally, previous studies have found a relationship between sample depth and the abundance of biotic alteration (Furnes et al., 2001a; Furnes and Staudigel, 1999). The authors found that up to 250 meters depth in the basaltic layer of the crust, biotic alteration was very common, and that it below this depth the abundance decreases.

Environmental parameter III. Sediment thickness

Overlying sediment thickness was chosen as a proxy for the amount of oxygenated seawater entering the basaltic layer (Table 2). Some sediment hydrologically isolates the basaltic layer at thicknesses over 200 meters (Stein and Stein, 1994). Outcrops, fractures systems, and any other features on the seafloor where seawater can be introduced are

buried and remaining fluids become less oxygenated over time since seawater can only enter locally (Stein and Stein, 1994).

Environmental parameter IV. Temperature

Temperature was selected because of the limited range in which microbial systems can survive (Harrison et al., 2013; Klonhauser, 2007). Common temperature tolerances have resulted in a division based on microbe type (Table 2). These are psychrophile (0-20°C), mesophile (20-24°C), thermophile (45-80°C), and hypothermophile (>80°C). The known temperature limit of life is currently 120°C (Kashefi and Lovely, 2003). Because temperature conditions are important for the survival of life, knowledge of the ambient temperatures will provide a better understanding of the mechanism for texture formation.

Using the same model of heat conduction as Heberling et al., 2010, (initially modeled by Stein and Stein, 1994), the temperature at the sediment basement interface was modeled as a function of age and distance between the warmer crust and the cold seawater (Appendix D). This distance is defined as the sediment thickness.

Some assumptions of this model include, (1) the upper oceanic crust is assumed to be a well-mixed aquifer where temperature is homogenized by circulation, (2) cold water enters the well-mixed aquifer and while flowing laterally warms before exiting the seafloor again (Fisher and Becker, 2000).

Also, previous work by Furnes et al., (2001a) suggested that temperature fluctuations between the near axis hydrothermal environment, (~350°C) (Mottl, 2003), and the colder, sealed crust prior to subduction (~160 Ma), might influence the subsurface biosphere in the ocean crust.

Environmental Parameter V. Secondary mineralogy

Secondary minerals (Table 2) are used in this study as proxies for the environmental conditions. If biotic textures are produced by microbial activity, secondary minerals can provide insight into what microbial metabolic processes are associated with the textures (Alt and Mata, 2000). For example, sulfur cycling is considered a likely candidate for

microbial metabolism in the subsurface (Bach and Edwards, 2003b; Lever et al., 2012). The presence of celadonite, which is indicative of oxygenated waters, can indicate if oxidizing conditions were present in the rock (e.g. Furnes & Staudigel 1999; Furnes et al., 2001a). Similarly, pyrite is an indicator of reducing conditions (Faure, 1998; Konhauser, 2007) and therefore can be used as a proxy to potentially infer sulfate reduction (Furnes et al., 2001a; Furnes and Staudigel, 1999; Lever et al., 2012).

Other examples of how conditions can be inferred using mineral proxies are for zeolite, carbonate, and smectite minerals. Zeolites are indicative of higher temperatures and high pH, also large amounts of dissolved silica that can become oversaturated (Faure, 1998). Carbonate minerals require a higher pH and low dissolved CO₂. Carbonate also is a material frequently colonized by microbes, and microborings similar to those in glass are observed in calcite brachiopod shells, and therefore the presence of carbonate indicates a viable environment, and also carbon source (Buijs, et al., 2004). Smectites were also identified by the drilling programs but broad equilibrium conditions prevent their utility here as a proxy.

Secondary mineralogy information was obtained from the ocean drilling program volumes; see Appendix E for example pages showing the method. Minerals linked to the exact sample location in the core were used. In the event a sample location was not associated with a mineral identification a mineral identification from a nearby sample was used. The maximum distance over which this was allowed to occur was one meter. The minerals recorded in the drilling program volume observations were, oxidized minerals (celadonite, iddingsite, limonite, oxyhydroxides), smectite, carbonate (calcite), and pyrite.

Reflected light microscopy was used to examine the thin sections for sulfide mineralization in the alteration assemblage. Sulfides were identified along fractures, and anywhere alteration or palagonite was present. Primary sulfides were also identified but were excluded if they were not spatially connected to biotic alteration. The most common location of sulfides associated with biotic textures was along fractures.

2.3 Treatment of data

2.3.1 Descriptive data analysis

The environmental parameters (Figure 24, Table 2, and Table 3) were divided into bins (Table 4) based on specific characteristics of the subseafloor and of biotic systems (Table 3 and Table 4). Secondly, an attempt was made to allocate a similar number of samples to each bin for each parameter (Figure 24, Table 3, and Table 4). Once the environmental parameters were binned, the samples in each bin were evaluated for the presence of the texture types.

The environmental parameter bins contain different sample amounts and so to compare between bins, only the relative proportion, or the percentage, of the respective textures and high abundance samples in each bin was used, not the absolute quantity of samples with the distinctive textures and high abundance. The bin that held the highest percent of samples for each respective texture type and high abundance was the focus of the descriptive analyses.

Table 3. Environmental parameter and abundance distribution of all samples

<i>I. Age Ma</i>	<i>Frequency</i>	<i>% of all samples</i>	<i>II. Sample depth into basalt (m)</i>	<i>Frequency</i>	<i>% of all samples</i>
0-20	32	40%	0-50	38	48%
21-40	18	23%	51-100	20	25%
41-60	10	13%	101-150	9	11%
61-80	4	5%	151-200	1	1%
81-100	2	3%	201-250	1	1%
101-120	7	9%	251-300	0	0%
121-140	0	0%	301-350	3	4%
141-160	7	9%	351-400	0	0%
			401-450	3	4%
			451-500	4	5%
			501-550	1	1%
<i>III. Sediment Thickness (m)</i>			<i>IV. Temperature (°C)</i>		
0-150	28	35%	≤10	10	13%
151-300	15	19%	10 < t ≤ 20	30	38%
301-450	14	18%	20 < t ≤ 30	15	19%
451-600	11	14%	30 < t ≤ 40	6	8%
601-750	3	4%	40 < t ≤ 50	3	4%
751-900	0	0%	50 < t ≤ 60	2	3%
901-1050	7	9%	60 < t ≤ 70	7	9%
1051-1200	0	0%	70 < t ≤ 80	1	1%
1201-1350	0	0%	80 < t ≤ 90	3	4%
1351-1500	2	3%	90 ≤	3	4%
<i>V. Secondary Mineralogy</i>			<i>**Abundance</i>		
Smectite	23	29%	0%	0	0%
			10%	0	0%
Carbonate (calcite)	55	69%	20%	1	1%
			30%	2	3%
Zeolites	15	19%	40%	7	9%
Oxidized minerals	18	23%	50%	9	11%
			60%	11	14%
Pyrite	5	6%	70%	13	16%
			80%	7	9%
*Sulfides	45	56%	90%	14	18%
			100%	16	20%

* Sulfide observations from thin sections. Other mineral observations were made on ship. **The percent of biotic alteration along glass surfaces: fractures, vesicles, and mineral grains. See Appendix F for graphs of these variables.

Table 4. Environmental parameter bins used for descriptive analysis with brief explanations of the bin divisions and the number of samples in each bin.

<i>I. Sample Age</i>		<i>Bin range (m)</i>	<i>Frequency</i>
• young	• Seawater basalt interaction phase*	≤20	32
• middle	• Intermediate	20 < age ≤65	30
• oldest	• Late stage seawater basalt interaction, anoxic conditions, loss of fluid circulation*	65 < age ≤160	18
<i>*Alt and Honnorez, 1984; Alt et al., 1986; Staudigel and Hart, 1981; Stein and Stein, 1994</i>			
<i>II. Sample Depth into Basalt</i>		<i>Bin range (m)</i>	<i>Frequency</i>
• shallow	• High porosity and permeability *	0 < D ≤50	38
• deep	• Homogenous alteration minerals, decrease in porosity and permeability*	50 < D ≤550	42
<i>*Alt and Honnorez, 1984; Alt et al., 1986; Furnes et al., 2001a; Furnes et al., 2002; Schrag et al., 1992</i>			
<i>III. Overlying Sediment Thickness</i>		<i>Bin range (m)</i>	<i>Frequency</i>
• moderate	• Open ocean, hydrothermal circulation enables heat transfer*	20 < sed. ≤300	44
• thick	• Continental margin, hydrologically isolated igneous crust due to sedimentation*	300 < sed. ≤ 1550	36
<i>*Parkes et al., 1994; Stein and Stein, 1994</i>			
<i>IV. Temperature</i>		<i>Bin range (°C)*</i>	<i>Frequency</i>
• low	• Psychrophile growth range	0 < T ≤20	40
• medium	• Mesophile growth range	20 < T ≤45	22
• high	• Thermophile, hyperthermophile growth range	45 < T ≤120	18
<i>*Gerday and Glansdorff, 2007; Konhauser, 2007; Michie, et al., 2011; Nedwell, 1999</i>			
<i>V. Secondary Mineralogy*</i>			<i>Frequency</i>
• Smectites	• Chlorite and smectite mineral observations were binned together to represent similar mineral stability conditions		23
• Oxidized minerals	• All oxidized minerals are grouped to account for all evidence of oxidized conditions		18
<i>Abundance of biotic alteration along fractures, varioles, vesicles, and anywhere accessible to seawater.</i>			
low	Lower abundance of biotic alteration of the total alteration.	<70%	30
high	The average abundance amount in this study is 70%, see section 3.1.1 for further discussion.	≥70%	50

Environmental Parameter I. Sample Age

The sample ages were divided among three bins young (≤ 20 Ma), middle ($20 < \text{age} \leq 65$ Ma), and old (> 65) (Table 4). Most of the sample ages in this study fall below 20 Ma (40%); the average sample age is 47 Ma (Table 3 and Figure 24). Increasing age of the crust has several influences on sample environment. For example, temperature decreases over time (Stein and Stein, 1994). Fluid flow influences the circulation of oxidants and nutrient transport, which decrease with age and the sealing of the crust. Fluid flow is also controlled by the amount of sediment deposited over time.

The division at 20 Ma was selected because of its close proximity to the ridge crest and for the large relative variation in sediment thicknesses near the ridge crest, as well as for the high level of hydrothermal fluid circulation near the active volcanic spreading center (Fisher, 1998; Stein and Stein, 1994). This division also separates the period of time prior to 65 Ma when heat flux and alteration are higher, due to fluid flow in the crust into two bins. The division of 65 Ma is the result of a difference between theoretical and measured heat flow that occurs in younger crust (Stein and Stein, 1994). Crust older than 65 Ma is thought to be sealed because the circulation of fluids is inhibited by minerals deposited in fractures, voids, and pore spaces, therefore samples older than 65 Ma were grouped together. After 65 Ma, fluid circulation is minimal due to reduced permeability and porosity because of mineral deposition, so heat transfer only occurs through conduction.

Environmental Parameter II. Sample depth into basalt

The division of samples into two bins based on the sample depth into the basaltic layer was shallow ($0 < \text{basalt depth} \leq 50\text{m}$), and deep ($50\text{m} < \text{basalt depth} \leq 550\text{ m}$) (Table 4). The average sample depth into basalt is 102 meters and the majority of the samples occur in the upper 50 meters (48% of 80) (Figure 24 and Table 3). This placed an approximately even number of samples in each bin, and allowed changes in biotic alteration to be examined in terms of changing permeability and porosity with depth. Permeability and porosity in turn control the amount of fluid circulation and therefore

oxygen and nutrient transport in a similar manner to the age of the crust. A basalt depth of 50 meters marks a shift in porosity, above this depth porosity is ~20%, and below this depth it decreases to ~1% by 1 km depth (Evans, 1994). Permeability decreases after 50 meters from $5 \times 10^{-12} \text{ m}^2$, to $\sim 3 \times 10^{-17} \text{ m}^2$ at 1 km depth (Evans, 1994). These values are modeled at the East Pacific Rise at site 504B. Another model showed that at depths below 50 meters the secondary mineralogy of the basaltic crust is relatively homogenous suggesting the physical and chemical conditions are homogenous (Alt and Honorez, 1984).

Environmental parameter III. Overlying sediment thickness

The division of samples based on the thickness of sediment overlying the drill site created a moderate sediment thickness (0-≤300 meters) category, and a thick sediment category, (300 to ≤1550 meters) (Table 4). The average overlying sediment thickness was 350 meters and about half of the samples (54%) occur in the upper 300 meters (Figure 24 and Table 3). These divisions were made because where there is less than several hundred meters of sediment deposition seawater can enter the crust readily, carrying oxygen and nutrients (Stein and Stein, 1994). After several hundred meters of sediment it has been proposed the igneous basement becomes hydrologically isolated from seawater and recharge can only occur from distant outcrops. The specific 300 meter division was selected because about half the samples fell in each bin in contrast to placing it at 200 meters. Overall exact value of the 300 meter division was intended to equalize the number of samples in each bin.

Environmental parameter IV. Temperature

The division of samples based on temperature, was made because of how microbial systems respond to changes in temperature (Table 4). The average temperature was 31°C and most the samples are more frequently in the colder temperature range, and decrease almost steadily in the higher ranges. Microbes capable of surviving at the temperatures reported in this study include: psychrophiles (~0-20°C) (low temperature), mesophiles (20-45°C) (intermediate temperature), thermophiles and hyperthermophiles (45-120°C)

(high temperature) (Gerday and Glansdorff, 2007; Michie, et al., 2011; Nedwell, 1999). Some living systems can tolerate a broader range of temperatures but these temperature tolerances are commonly documented average values for most species.

Environmental Parameter V. Secondary Minerals

The secondary minerals were discrete variables that did not require binning in the same manner as the continuous variables such as age and depth (Table 4). The most often occurring mineral was carbonate followed by sulfides (Table 4). The binning that occurred was to insure that minerals with similar equilibrium conditions were grouped together. For example, the oxidized minerals, limonite, iddingsite, oxyhydroxides, celadonite, were grouped together. Chlorite was incorporated into the smectite mineral counts. The biotic alteration could then be considered in terms of the same type of environmental conditions as indicated by the secondary minerals, e.g., oxidizing conditions for oxidized minerals.

2.3.2 Quantitative data analysis

The focus of these analyses was to determine whether a correlation exists between biotic alteration and the environmental parameters associated with the rocks. Analyses were also conducted focusing on the association between abundance and distinctive textures. Two simple correlation coefficients, Pearson's and Spearman's, were selected to determine the extent and significance of any correlations (Appendix F). The results were then used to draw the final conclusions in this study. The method used to obtain the significance measures is described below and the results are given in detail in Appendix F.

There are several correlation coefficients commonly used to determine the strength of a relationship between two variables (Elith and Leathwick, 2009; Swan and Sandilands, 1995). The sample correlation coefficient r is appropriate to explore the underlying influence of geologic processes where little is known about the relationship between variables (Swan and Sandilands, 1995). This study falls into this category as the

effect of environmental variables on biotic alteration is still in the exploratory phase (Furnes et al., 2008; Knowles et al., 2013; Staudigel et al., 2008) and the microbial community composition remains undefined (Lever et al., 2013).

Correlation coefficients measure the significance two values assume within the same sample (Zar, 1999). Two of these, Spearman's rank correlation coefficient (r_s) and Pearson's product moment correlation coefficient (r_p) were chosen as exploratory measures of the variation between parameters. Pearson's does not demonstrate a relationship between variables; rather it demonstrates the tendency for the two variables to form a straight line (Swan and Sandilands, 1995; Zar, 1999). Pearson's r and Spearman's r correlation coefficients were calculated using IBM SPSS Statistics v. 16.0.

Spearman's method is non-parametric and converts every observation into rank and then finds the correlation of the ranks (Currell and Dowman, 2009; Swan and Sandilands, 1995). A perfect correlation results in a value of 1 for both Pearson's r_p and Spearman's r_s , and both vary between 0 and positive or negative 1 depending on the strength and sign of the correlation. The closer the value of the coefficients are to 0 the weaker the correlation. For Pearson's r_p a perfect 1 indicates that there is a tendency of the two variables to form a line, and for Spearman's r_s a perfect 1 indicates that the two variables either increase or decrease monotonically. The analysis is designed to measure the strength of the monotonic relationship between two measurements and is obtained as follows (Currell and Dowman, 2009; Steel and Torrie, 1980; Zar, 1999).

The analyses also served to overcome any loss of detail during the environmental analyses when the continuous parameters, sample age, sample depth, overlying sediment thickness, and temperature were turned into discrete values in the bins. During these correlation tests these variables were left continuous.

Three hypotheses were chosen to test the correlations between the textures and the parameters, the abundance and the parameters, and the textures and the abundance. Three null hypotheses were therefore also created. A significant correlation will indicate there is either a less than 1% chance ($P < 0.01$) or less than 5% chance ($P < 0.05$) the result would

be produced if the null hypothesis is true. It does not indicate whether the hypothesis is supported it indicates the probability the null hypothesis is not supported.

Hypothesis A

H_{1A} Biotic alteration textures (9) granular, mossy, overprinting, empty, thin, convoluted, dark, curvilinear, long, vary with the environmental parameters sample age, sample depth, overlying sediment thickness, temperature, and the secondary mineralogy.

- For Pearson's r_p to be selected the distribution of the data has to be normal. If this is not the case, Spearman's r_s , a non-parametric correlation coefficient, is more appropriate (Currell and Dowman, 2009; Zar, 1999). The four continuous environmental parameters, sample age, sample depth into basalt, overlying sediment thickness and temperature, indicated strong positive skew with respect to a normal distribution. Therefore, Spearman's r_s was used in the correlations conducted for the four continuous environmental parameters.
- To increase the statistical significance of these analyses the environmental values, sample age, depth into basalt, overlying sediment thickness, temperature, were not binned resulting in a population of $n=80$. While this did not allow for comparison between the binned environmental data maximum and minimum occurrence values (method described in Section 2.3.1 above), the robustness of the statistical significance was improved.
- Pearson's was used to correlate between the distinctive textures and the secondary mineralogy.

Null hypothesis A

H_{0A} Biotic alteration textures (9) granular, mossy, overprinting, empty, thin, convoluted, dark, curvilinear, long, does NOT vary with the environmental parameters sample age, sample depth, overlying sediment thickness, temperature, and the secondary mineralogy.

Hypothesis B

H_{1B} Biotic alteration abundance amount as a percent of the total alteration present along glass surfaces varies with the environmental parameters, sample age, sample depth, overlying sediment thickness, temperature, and the secondary mineralogy.

- Biotic alteration abundance illustrated negative skew with respect to a normal distribution. Therefore, Spearman's r_s was used in the correlations conducted for the four continuous environmental parameters.

Null hypothesis B

H_{0B} Biotic alteration abundance amount as a percent of the total alteration present along glass surfaces does NOT vary with the environmental parameters, sample age, sample depth, overlying sediment thickness, temperature, and the secondary mineralogy.

Hypothesis C

H_{1C} Biotic alteration textures (9), granular, mossy, overprinting, empty, thin, convoluted, dark, curvilinear, long, vary with the biotic alteration abundance as a percent of the total alteration present along glass surfaces.

- Biotic alteration abundance illustrated negative skew with respect to a normal distribution. Therefore, Spearman's r_s was used in the correlations conducted for the four continuous environmental parameters.

Null hypothesis C

H_{0C} Biotic alteration textures (9), granular, mossy, overprinting, empty, thin, convoluted, dark, curvilinear, long, do NOT vary with the biotic alteration abundance as a percent of the total alteration present along glass surfaces.

3. RESULTS

To test my hypothesis that biotic alteration of subseafloor volcanic glass samples varies with the *in situ* sample environment, the amount of biotic alteration, and the biotic alteration textures were documented (Section 3.1) and compared to the sample environment conditions (Section 3.2). The presence of the biotic alteration textures and high abundance was investigated to determine where the maximum number of samples containing each of the biotic alteration characteristics occurred, e.g., what sample age's mossy texture occurred with most frequently. These were called the maximum occurrences, or conversely the minimum occurrences.

These results were supplemented by the calculation of correlation coefficients to evaluate the significance of the occurrence of biotic alteration with the parameters (Section 3.3). Verification of the coefficient results are presented in this section, and in the discussion section where relevant, and in Appendix F.

The 80 samples come from 67 unique locations (Appendix C). There are some disadvantages and some advantages to selecting samples from the same location and samples from multiple locations. The samples from multiple locations capture a broader geographic range and the samples from the same locations could be affected by anomalous local environmental conditions that are actually not representative of the whole oceanic crust. Because this study is intended to investigate a global sample set, a higher number of sample locations is considered optimal.

3.1 Biotic alteration

3.1.1 Biotic alteration abundance

Biotic alteration abundance was estimated as a percentage of the total alteration present in a sample. Estimates of biotic alteration that occur along linear features such as fractures, grain boundaries, vesicle edges, varioles and other quench features were made in increments of 10% (Figure 10 and Table 4). For example, the lowest abundance bin

was 0% biotic alteration; the next was 10% biotic alteration, then 20%, on up to 100% biotic alteration out of the total alteration.

The division of biotic alteration abundance was intended to capture a large number of samples to compare with the environmental parameters (Figure 24, Table 3, and Table 4). The average, and the median, abundance quantity for the 80 samples was 70% and this served as the basis for the division into high and low abundance. The division was also based on abundance studies conducted by Cousins (2009) and Furnes and Staudigel (1999).

Several studies have found correlations between biotic alteration abundance and environmental conditions. Cousins (2009) found that abundance is influenced by the source of fluids: glacial melt water versus seawater. In samples that are predominately marine, between 60 and 100% biotic alteration is common in contrast to fresh water samples. Furnes and Staudigel (1999) found that biotic alteration abundance varies between 55% and 90% in samples less than 200 meters depth in the Atlantic Ocean. Overall 75% is the average abundance amount for depths less than 250 meters in the Atlantic Ocean (Furnes and Staudigel, 1999; Furnes et al., 2001a). These studies suggest that samples with over 50% abundance are related to environmental characteristics. This difference between abundance values over 50% and less than 50% biotic alteration therefore served as a starting point for investigation.

The division of samples containing over 50% biotic alteration is used to bin the samples for comparison. Initially, because of previous studies, e.g., Cousins, 2009; Furnes and Staudigel, 1999; Furnes et al., 2001a, 50% and above was selected to divide the samples (Figure 10). This resulted in a high number of samples in the greater than 50% category. When the division was set at 60% 61 samples were placed in the high abundance category and 19 samples in what would be called the low abundance category (< 60% biotic alteration) (Figure 24 and Table 3). However, an evaluation of how the data were distributed in the abundance amounts with an 80% bin division revealed that 80% is an anomalous abundance amount that deviated from the trend of increasing

numbers of samples as abundance increased (Figure 10). To prevent the deviation in the 80% abundance set from affecting the analyses, the abundance division was set at 70%.

The samples were divided into two categories based on biotic alteration abundance (Table 4 and Figure 10). Samples with *greater than or equal to 70%* biotic alteration are said to have *high abundance*, while samples with *less than 70%* biotic alteration are said to have *low abundance* (Figure 10). With this division, 50 of the 80 samples contain over 70% biotic alteration. High abundance of biotic alteration is more common than low abundance, and most samples contain >50% biotic alteration (Table 3 and Figure 10). The most frequent biotic alteration abundance increment is 100% biotic alteration. The category with the fewest number of samples is 20% biotic alteration abundance which included only one sample. All samples contain biotic alteration, the 0 and 10% abundance increments were not observed in any samples (Table 3 and Figure 10).

With the exception of the 80% abundance bin the number of samples increases consistently with the abundance of biotic textures (Table 3 and Figure 10). The mode was 100% abundance, occurring in 16 of the samples, or 20% (Table 3). Samples with over 50% biotic alteration comprise almost 90% of the samples (Table 3 and Figures 10).

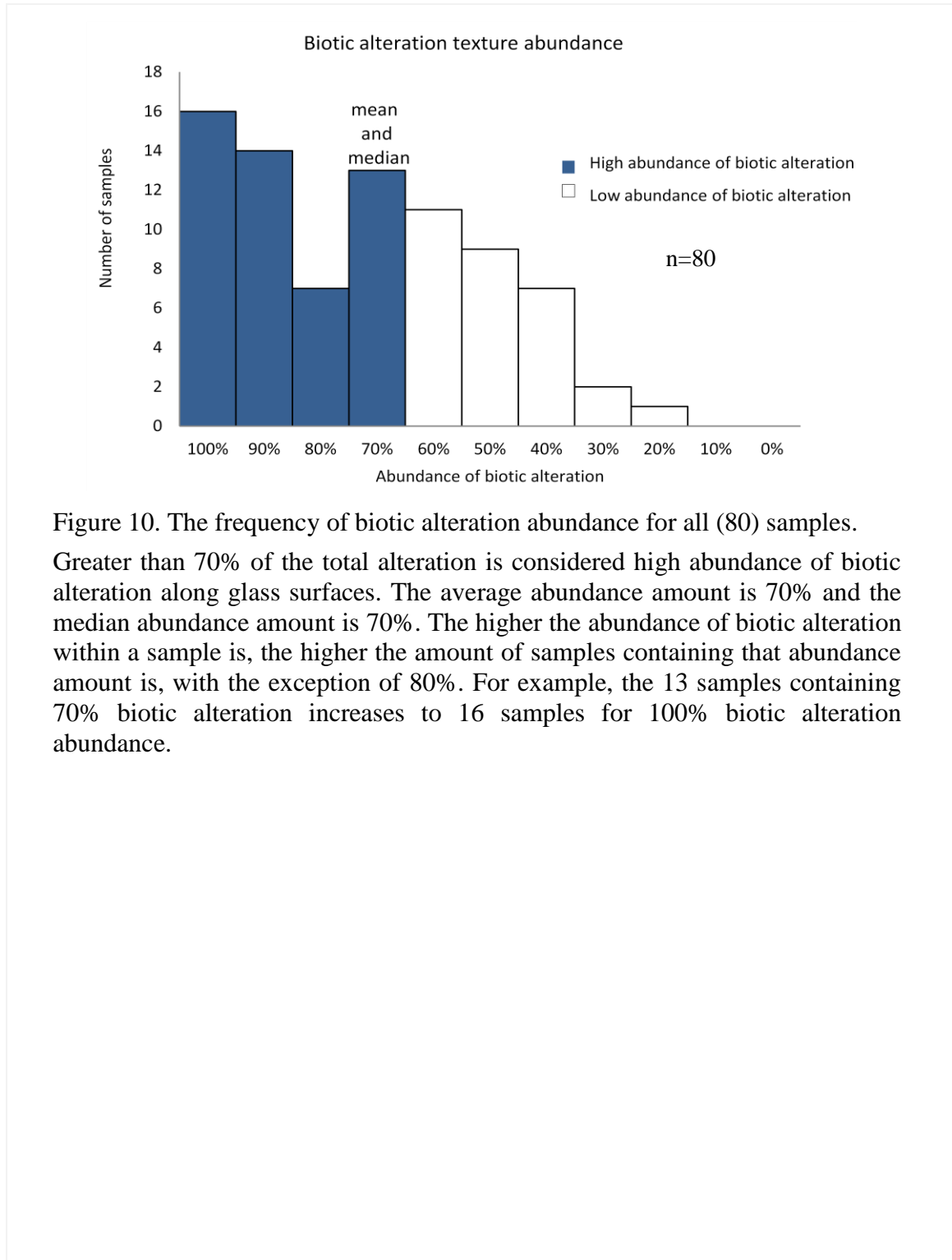


Figure 10. The frequency of biotic alteration abundance for all (80) samples.

Greater than 70% of the total alteration is considered high abundance of biotic alteration along glass surfaces. The average abundance amount is 70% and the median abundance amount is 70%. The higher the abundance of biotic alteration within a sample is, the higher the amount of samples containing that abundance amount is, with the exception of 80%. For example, the 13 samples containing 70% biotic alteration increases to 16 samples for 100% biotic alteration abundance.

3.1.2 Distinctive biotic textures

Whenever there is altered glass the sample invariably contains both biotic and abiotic textures (Figure 3). Some biotic textures occur more frequently than others and many contain more than one texture type (Appendix C). Textures are distributed along fractures, vesicles, and at the edge of the sample. To a lesser degree, textures occur in association with varioles (quench feature). Biotic texture distribution varies within a sample, often with more than one texture type along a fracture or other region. However, some samples contain only one biotic texture type (Appendix C).

The most common texture types are granular, overprinting, mossy, empty, thin, convoluted, curvilinear, dark, and long, which all occur in over 30% of the samples, or at least 29 out of 80 total samples (Figure 8 and Figure 9). The most common texture type is granular, found in 74 of 80 samples (93%). The least common texture types are separate, simple, and engorged, which occur in only 2 out of the 80 samples (3%).

3.1.3 Distinctive biotic textures and high abundance

The relative proportion of high and low distinctive texture presence in samples remains consistent for samples with high abundance (Figures 9, and 10). For example, the most common texture type, granular, is found in 93% of the 80 samples (Figure 9), also occurs more frequently with high abundance than any of other distinctive textures (Figure 11). Overprinting occurs frequently in samples with high abundance, 80% (Figure 11) and it occurs in 71% of all 80 samples (Figure 9). This high frequency occurrence of overprinting and high abundance suggests a correlation. This correlation between overprinting and high abundance is corroborated with a significant positive correlation ($r_s = 0.24$) at the 95% confidence level (Table 7).

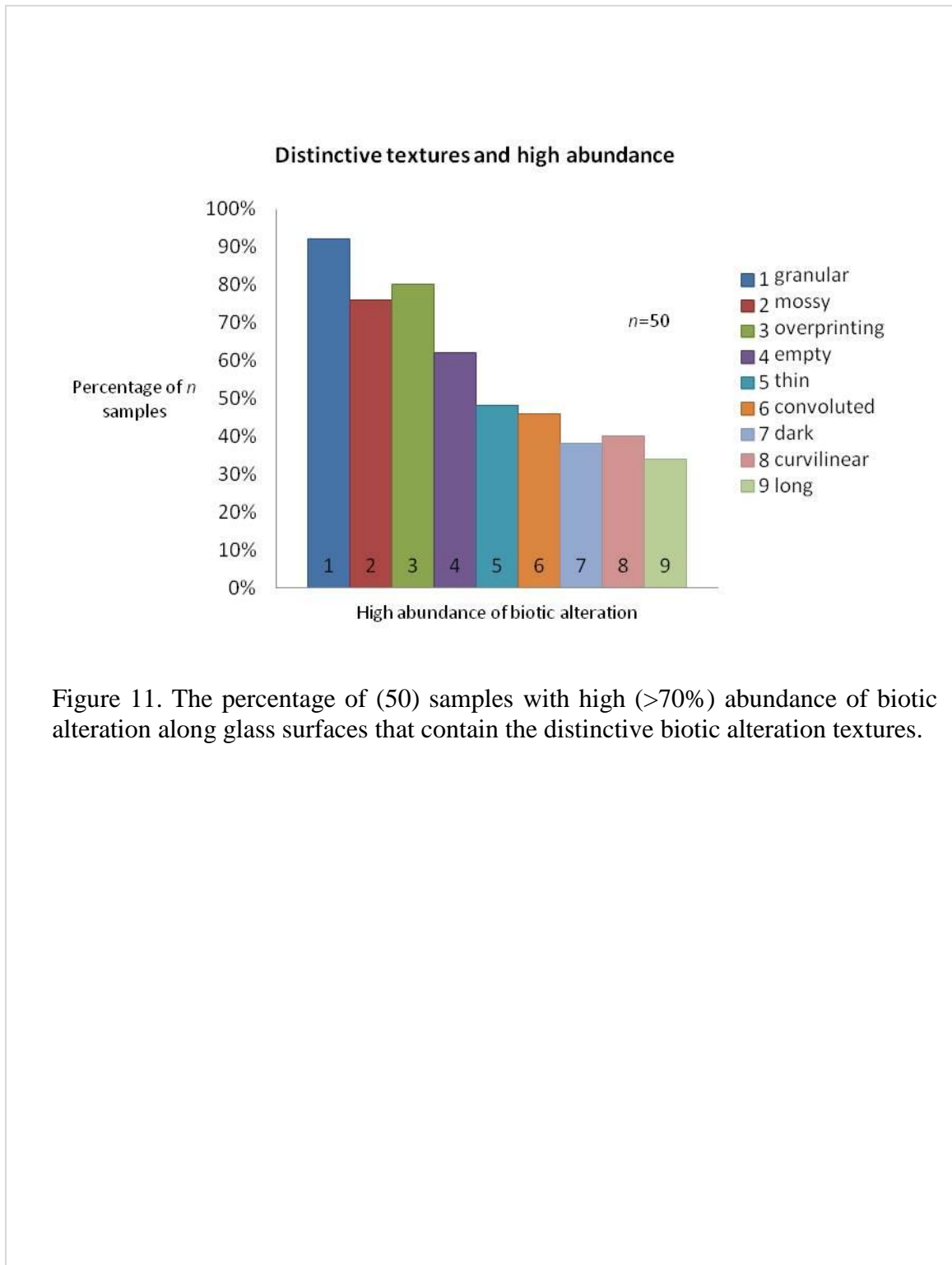


Figure 11. The percentage of (50) samples with high (>70%) abundance of biotic alteration along glass surfaces that contain the distinctive biotic alteration textures.

3.2 Environmental parameters

The six environmental parameters were divided into subgroups to identify how the texture types vary with change in the environmental parameters (Table 4). The selection process for the subgroups or divisions of environmental parameter values had two main objectives (Table 4). The first objective was to allocate an approximately equal number of samples to each bin. The second objective was to select environmental parameter bins that reflect different conditions in the crust and especially those that could impact biotic systems.

The environmental parameter bins contain differing sample amounts and so to compare between the bins, e.g., high versus low, only the relative proportion of the respective textures and high abundance samples in the bins were used, not the absolute quantity of samples in each bin. For example, the number of samples with granular texture in each sample age group was divided by the number of samples in that age group.

3.2.1 Biotic alteration and environmental parameters

The results of the biotic alteration and environmental parameter investigation are presented with the abundance correlations for each parameter, followed by the correlations between the distinctive textures and the parameters. The results show general trends and which environmental parameter bins have high abundance of biotic alteration and the distinctive textures in the largest number of samples. This is called *the maximum occurrence* of high abundance or the distinctive textures, e.g., if mossy occurs most frequently in older samples or younger samples.

Environmental parameter I: Sample Age

Measurements of sample age were obtained from the DSDP or ODP, and were compiled by Josef (2006) from the DSDP and the ODP volumes. Sample age was divided into three bins, ≤ 20 Ma, 20 to 65 \leq Ma, and ≤ 160 Ma (Table 5).

High abundance and sample age

High abundance samples occur more frequently in the older sample age bins (Figure 12). The number of samples in the two younger age bins with high abundance is almost equal; 56% of samples containing high abundance in the ≤ 20 Ma bin, and 60% of samples for the middle age bin. The oldest age bin, (>65 Ma) contains the highest percentage, 78%, of high abundance samples. High abundance of biotic alteration relates to sample age, and the higher frequency of biotic alteration in older samples suggests that biotic alteration continues in rocks older than 65 Ma.

Distinctive textures and sample age

The percentage of samples containing distinctive textures increases with sample age for six of the nine distinctive textures (Figure 13). Specifically, granular, mossy, overprinting, empty, thin, and long textures occur more frequently in the two older age bins. Granular texture occurs in over 91% of samples in the ≤ 20 Ma bin, and increases to 100% for the oldest age group. Mossy texture is found in 75% of samples within the first 20 Ma, and increases to 79% in the >65 Ma sample age bin. Similarly, overprinting and empty textures occur in more than half the samples in the young age bin, ≤ 20 Ma; 66% and 56% respectively. Overprinting and empty occur in 83% and 67% of samples in the oldest age bin, >65 Ma. The largest net change in abundance percentages occur for curvilinear textures. Curvilinear tunnels occurrence increases by 28% between the young age bin and the oldest age bin. The number of samples containing all the distinctive textures is highest in the oldest age bin (>65 Ma), which suggests that age has an effect on distinctive texture formation.

Samples with dark contents decrease from 47% (20 to ≤ 65 Ma) to 33% of the total number of samples in the oldest sample bin (Figure 13). Dark contents therefore decrease with age; this however does not indicate a decrease of any particular tunnel type, just the dark contents within the tunnels. This statement is important because the relationship between dark contents and tunnel formation is not known other than the dark contents are

deposited when the tunnel was formed or after the tunnel was formed because the dark contents are always found inside the tunnels (Figure 3 and Figure 8).

Sample age and distinctive texture formation are related. This was substantiated for curvilinear samples. An increase in curvilinear occurrence with increasing sample ages is significantly correlated ($r_s = 0.25$, $P < 0.05$) using Spearman's rank, at the 95% confidence level (Appendix F).

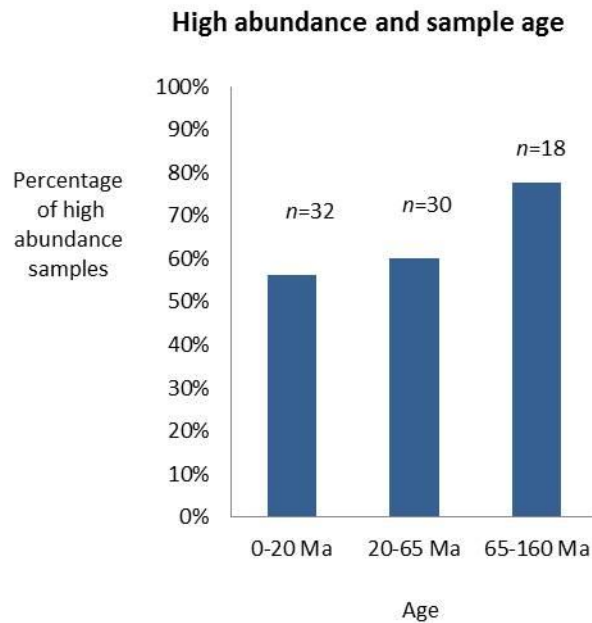
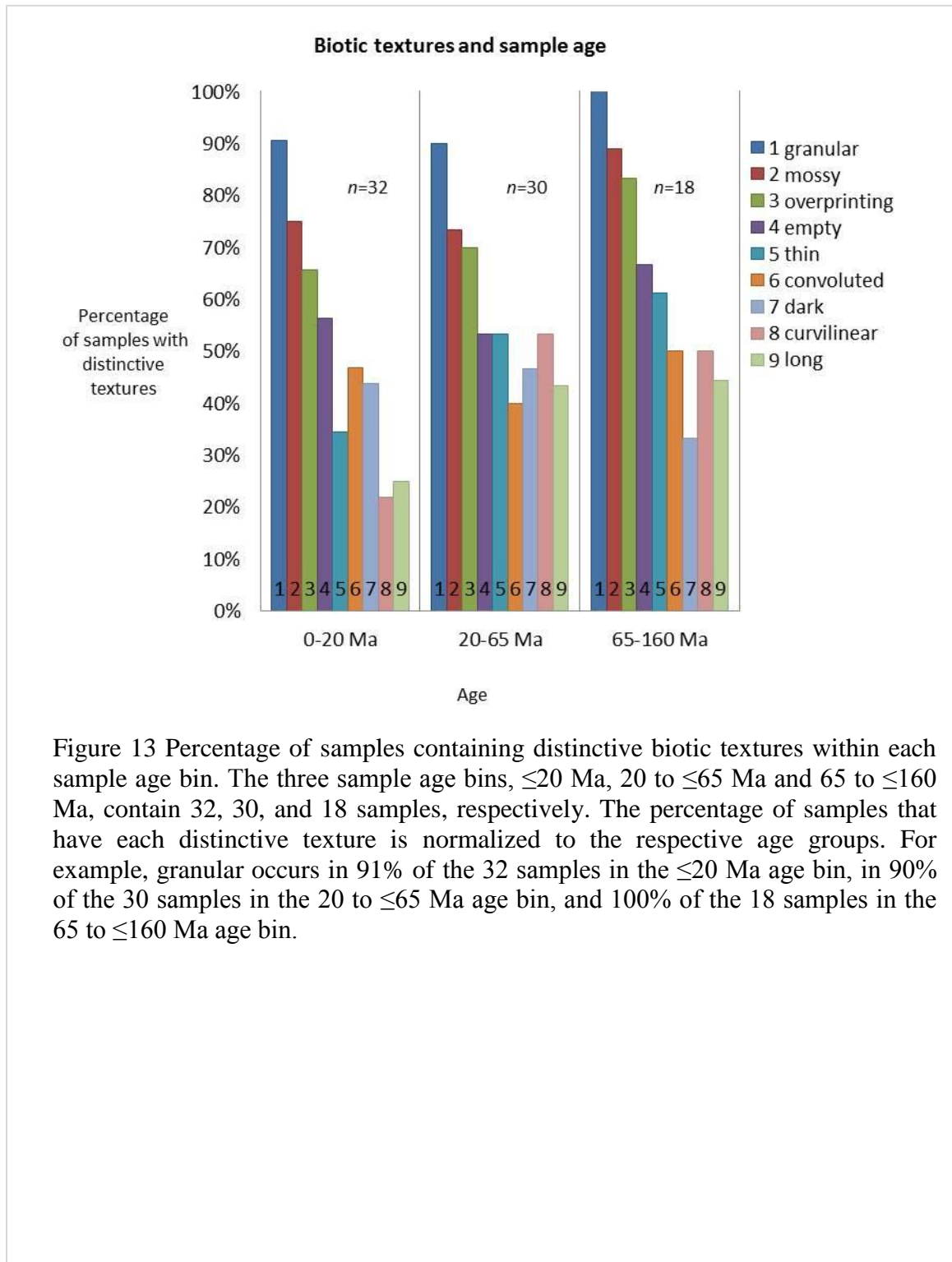


Figure 12. Percentage of high abundance samples within each sample age bin. High abundance of biotic texture is defined as $\geq 70\%$ of the alteration is biotic. Abundance is estimated along linear features in the sample such as fractures, vesicles, grain boundaries, and quench features. The three sample age bins, 0 to ≤ 20 Ma, 20 to ≤ 65 Ma and > 65 Ma, contain 32, 30, and 18 samples, respectively. The number of samples with high abundance in these age bins is normalized to the number in the bin. For example, of the 32 young, 30 middle and 18 old age samples, 56%, 60%, and 78%, respectively, contain a high abundance of biotic alteration.



Environmental parameter II: Sample depth into basalt

Sample depth measurements were made when the sample was collected by either the DSDP or the ODP. The depth measurements were compiled by Josef (2006) from the DSDP and the ODP volumes (Appendix C).

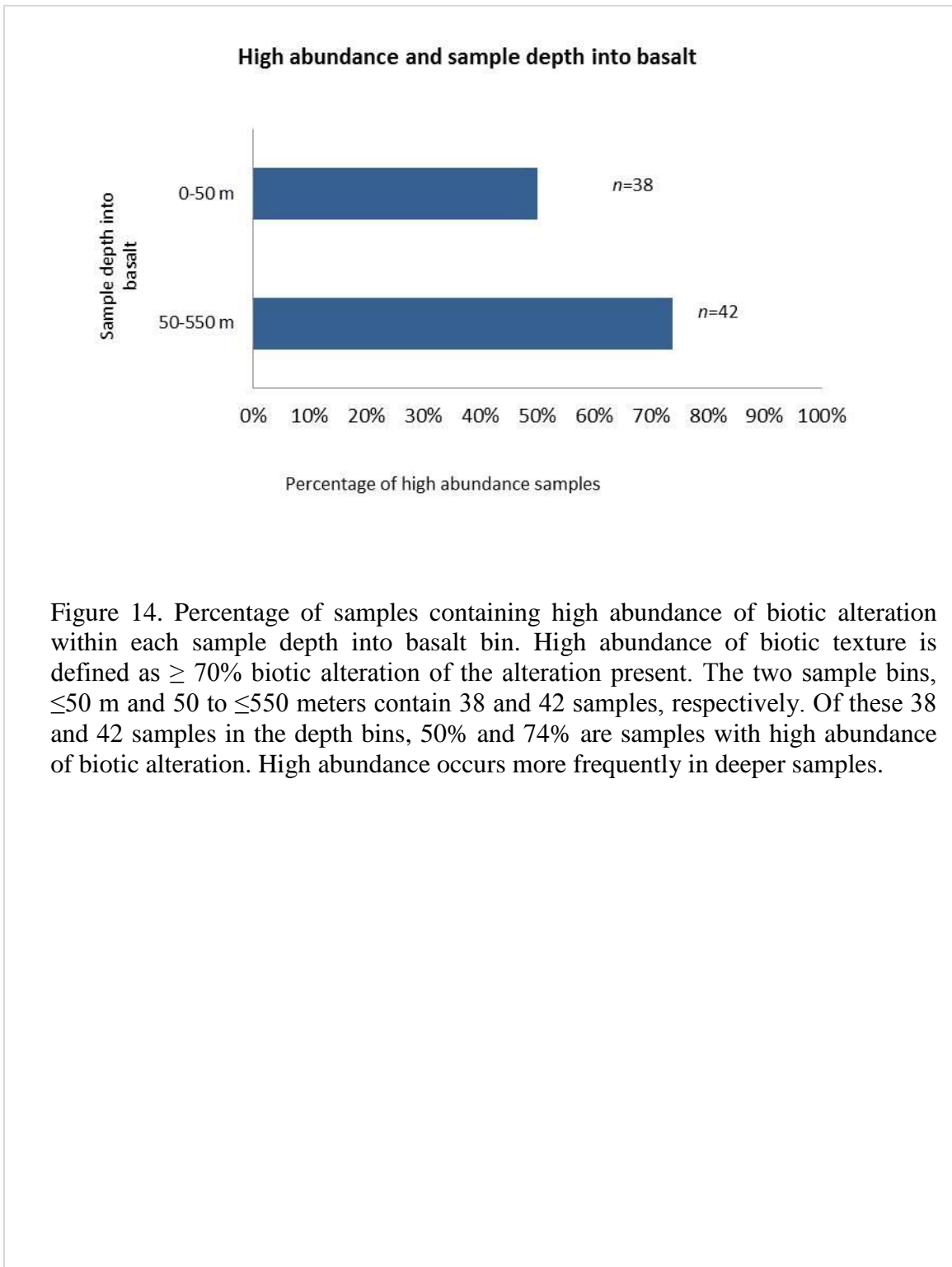
High abundance and sample depth into basalt

The percentage of samples with high abundance increases from (50%) for shallow samples, 0 to ≤ 50 meters of basalt, (Figure 14), to 74% of samples in the 50 to ≤ 500 meter sample depth bin. An increase in the percentage of samples with high abundance suggests that deeper in the crust, conditions are favorable for the formation of biotic alteration. These results are substantiated with a correlation between sample depths and high abundance using Spearman's correlation, ($r_s = 0.27$, $P < 0.05$) (Appendix F).

Distinctive textures and sample depth into basalt

Sample depth affected four of the nine distinctive textures, but only one of them, overprinting, shows a marked difference (Figure 15). Samples with overprinting, thin, and convoluted tunnels increase by over 10% with depth (Figure 15). The largest net change was for overprinting which increases from 58% of samples from 0 to ≤ 50 meters, to 83% of samples in the 50 to ≤ 550 meter sample depth range. Granular and curvilinear textures have a $\sim 1\%$ increase; mossy, empty, long, and dark textures increase $\sim 6\%$. This suggests sample depth does not impact distinctive texture formation. Since depth is a proxy for permeability, and the circulation of oxygenated seawater with nutrients, perhaps the microbes involved in texture formation are not influenced by changes in the oxidative state and can survive without a continued input of nutrients.

Sample depth has an effect on only one distinctive texture, overprinting, substantiating the lack of relationship between sample depth and distinctive textures. Overprinting and sample depth increase were found to have a statistically significant correlation at the 99% confidence level, using Spearman's correlation ($r_s = 0.29$, $P < 0.05$) (Appendix F).



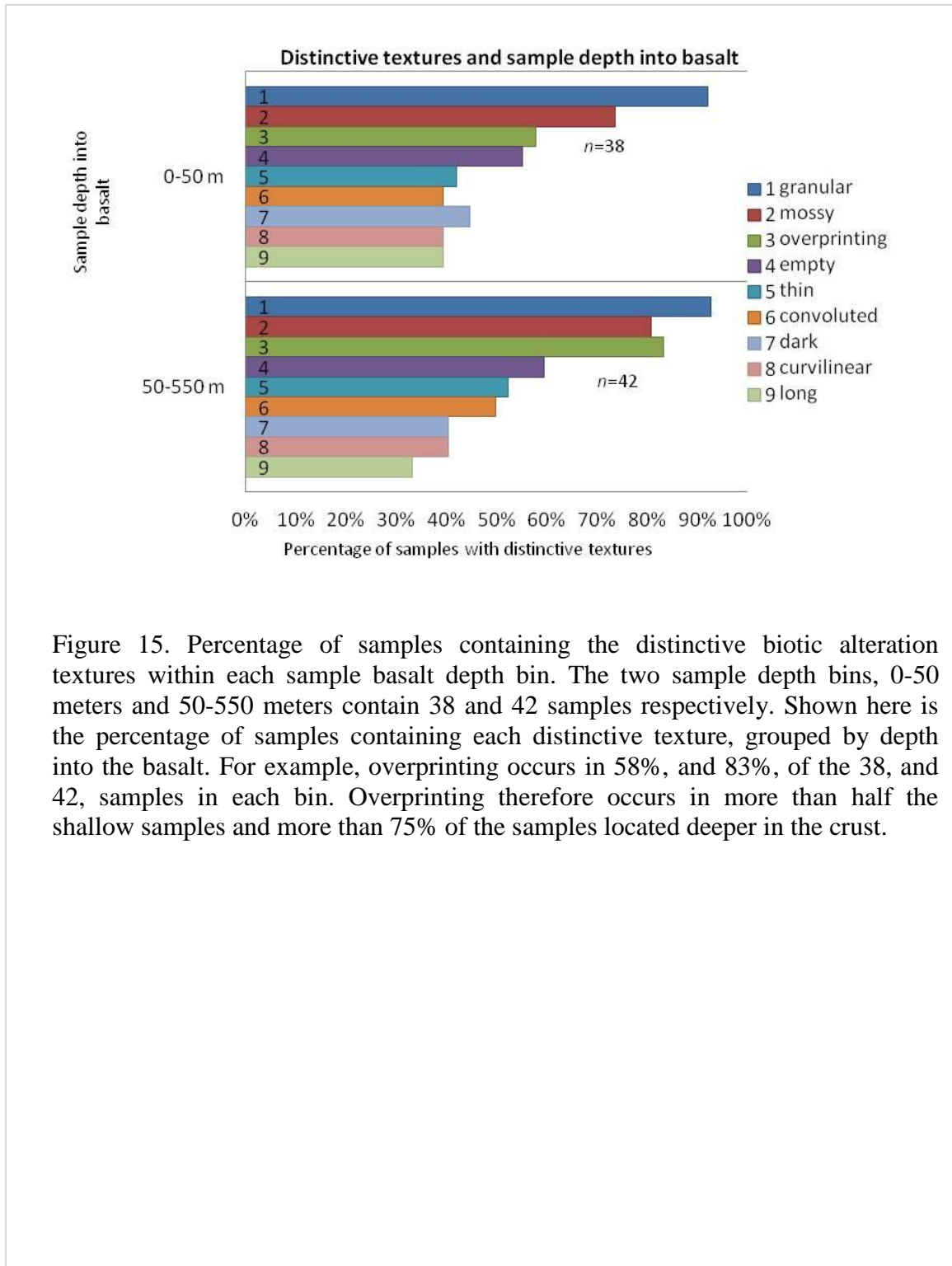


Figure 15. Percentage of samples containing the distinctive biotic alteration textures within each sample basalt depth bin. The two sample depth bins, 0-50 meters and 50-550 meters contain 38 and 42 samples respectively. Shown here is the percentage of samples containing each distinctive texture, grouped by depth into the basalt. For example, overprinting occurs in 58%, and 83%, of the 38, and 42, samples in each bin. Overprinting therefore occurs in more than half the shallow samples and more than 75% of the samples located deeper in the crust.

Environmental parameter III: Depth of overlying sediment thickness

Sediment thickness was measured when the samples were collected by the DSDP or the ODP and the values were compiled by Josef (2006) from the DSDP and the ODP volumes.

High abundance and overlying sediment thickness

High abundance samples do not show a marked change ($>10\%$) with thicker sediment (Figure 16). The percentage of samples with high abundance increases from 59% in ≤ 300 meters of sediment, to 67% in sediment thicker than 300 meters (Figure 16). Abundance does not seem to be significantly correlated with an increase in the depth of sediment deposited on the crust (Appendix F).

Distinctive textures and overlying sediment thickness

The largest change in percentage of samples containing the distinctive textures between the moderate sediment bin (≤ 300 m) and the deeper sediment bin (> 300 m), is for mossy and curvilinear textures (Figure 17). Mossy textures increase by 10%, with the change from the moderate to thick sediment bin, while curvilinear samples decrease from 45% to 33% from the moderate to the thicker sediment bin. Thin tunnels show the least change, a 1% decrease with the highest sediment thickness bin. For most texture types, change in sediment thickness was not correlated with a change in the number of samples containing distinctive texture types. Because sediment thickness reduces the influx of seawater at depths greater than several hundred meters, the lack of a relationship could suggest that biotic texture formation is unrelated to oxygenated seawater circulation.

The results of Spearman's rank correlation show *no correlation* between increasing overlying sediment thickness and any of the distinctive textures, substantiating the idea that sediment thickness does not influence the proliferation of biotic alteration. This indicates the conditions that are conducive to biotic alteration are perhaps related entirely to the basaltic crust or, there is some other environmental characteristic influencing the texture distribution.

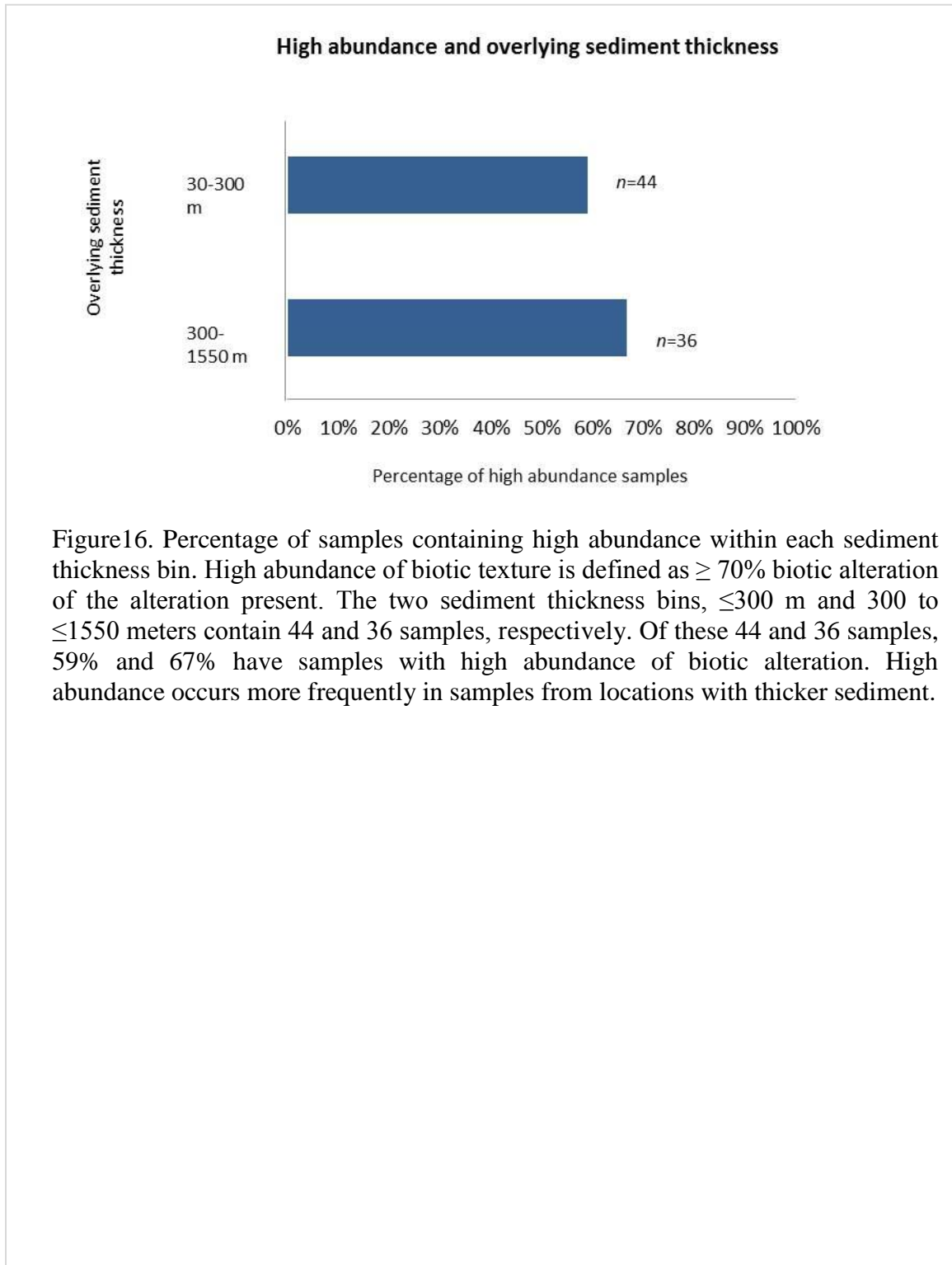


Figure16. Percentage of samples containing high abundance within each sediment thickness bin. High abundance of biotic texture is defined as $\geq 70\%$ biotic alteration of the alteration present. The two sediment thickness bins, ≤ 300 m and 300 to ≤ 1550 meters contain 44 and 36 samples, respectively. Of these 44 and 36 samples, 59% and 67% have samples with high abundance of biotic alteration. High abundance occurs more frequently in samples from locations with thicker sediment.

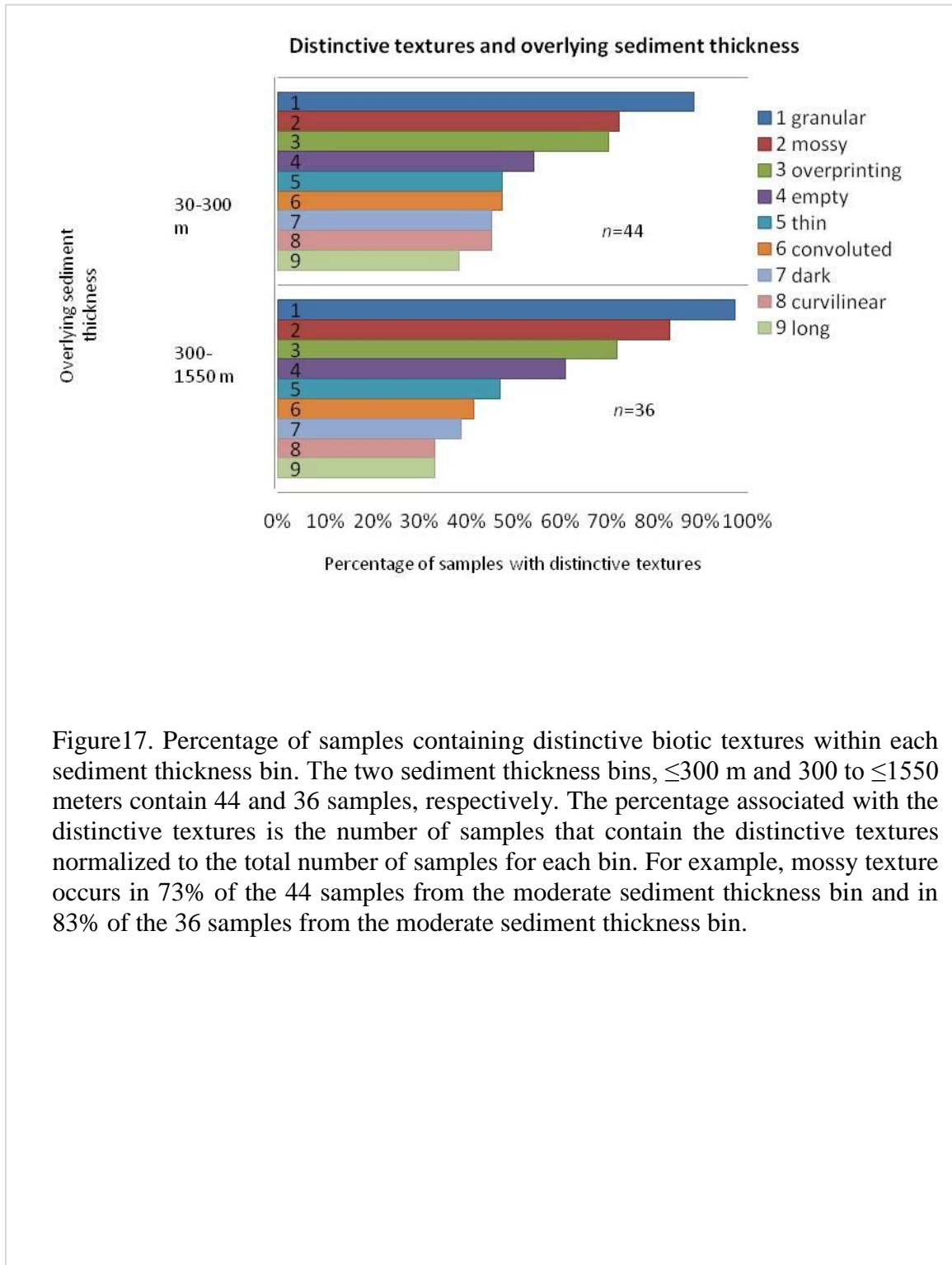


Figure17. Percentage of samples containing distinctive biotic textures within each sediment thickness bin. The two sediment thickness bins, ≤ 300 m and 300 to ≤ 1550 meters contain 44 and 36 samples, respectively. The percentage associated with the distinctive textures is the number of samples that contain the distinctive textures normalized to the total number of samples for each bin. For example, mossy texture occurs in 73% of the 44 samples from the moderate sediment thickness bin and in 83% of the 36 samples from the moderate sediment thickness bin.

Environmental parameter IV: Temperature

Temperatures were modeled as a function of sediment thickness and age, using a one-dimensional conductive cooling model in a half-space, after Heberling et al., (2010) and Stein and Stein (1994), see Appendix C.

High abundance and temperature

The percent of samples with high abundance of biotic alteration is 63% in the psychrophile temperature bin (0 to $\leq 20^{\circ}\text{C}$), 45% of the samples in the mesophile temperature bin (20 to $\leq 45^{\circ}\text{C}$), and 83% of samples in the $>45^{\circ}\text{C}$ temperature bin (thermophile and hyperthermophile) (Figure 18). This suggests that biotic alteration occurs at all temperature ranges and that higher temperature ranges, have the largest effect on the abundance of biotic alteration (Figure 19). This, however, is not substantiated using Spearman's rank where temperature is allowed to increase continuously. The lack of correlation could be the result of the small sample size, or because the bins were divided with half the samples in the youngest bin to account for the microbial tolerances, which resulted in only a quarter of the samples in the highest temperature bin.

Distinctive textures and temperature

The percent of samples containing the distinctive textures increases for mossy and dark textures, but decreases for granular, thin, convoluted, and curvilinear textures in the higher temperature bins (Figure 19). Samples with thin tunnels, curvilinear tunnels and convoluted tunnels textures occur most frequently in the lowest temperature bin, in 55%, 45% and 50% respectively, of samples. Granular, empty and long tunnels textures occur most frequently in the middle temperature bin, in 95%, 64%, and 41%, respectively, of the samples. Overprinting and dark tunnels occur most frequently in the highest temperature bin, in 83% and 50% respectively, of the samples in that temperature bin. Overprinting shows an increased occurrence for the highest temperatures; empty and granular textures occur more frequently in the middle temperature bin. This shows a lack

of a clear temperature correlation using the bin method for all of the distinctive textures, suggesting that the individual distinctive textures could be created by organisms with varying temperature tolerances. Alternative explanations could include that certain textures are formed at lower temperatures and as the sample heats, the textures are preserved.

The texture type with the only significant correlation with temperature, was curvilinear, which showed with a 95% confidence level, a negative Spearman's rank correlation of ($r_s = 0.23$) (Appendix F). This supports the idea that curvilinear textures are created by organisms that tolerate low temperatures.

Temperature was quantitatively correlated with only one of the distinctive texture types. This suggests that the microbes creating the textures have a wide temperature tolerance or that temperature has no influence on the occurrence of biotic alteration. Direct temperature measurements could provide insight into how temperature relates to biotic alteration.

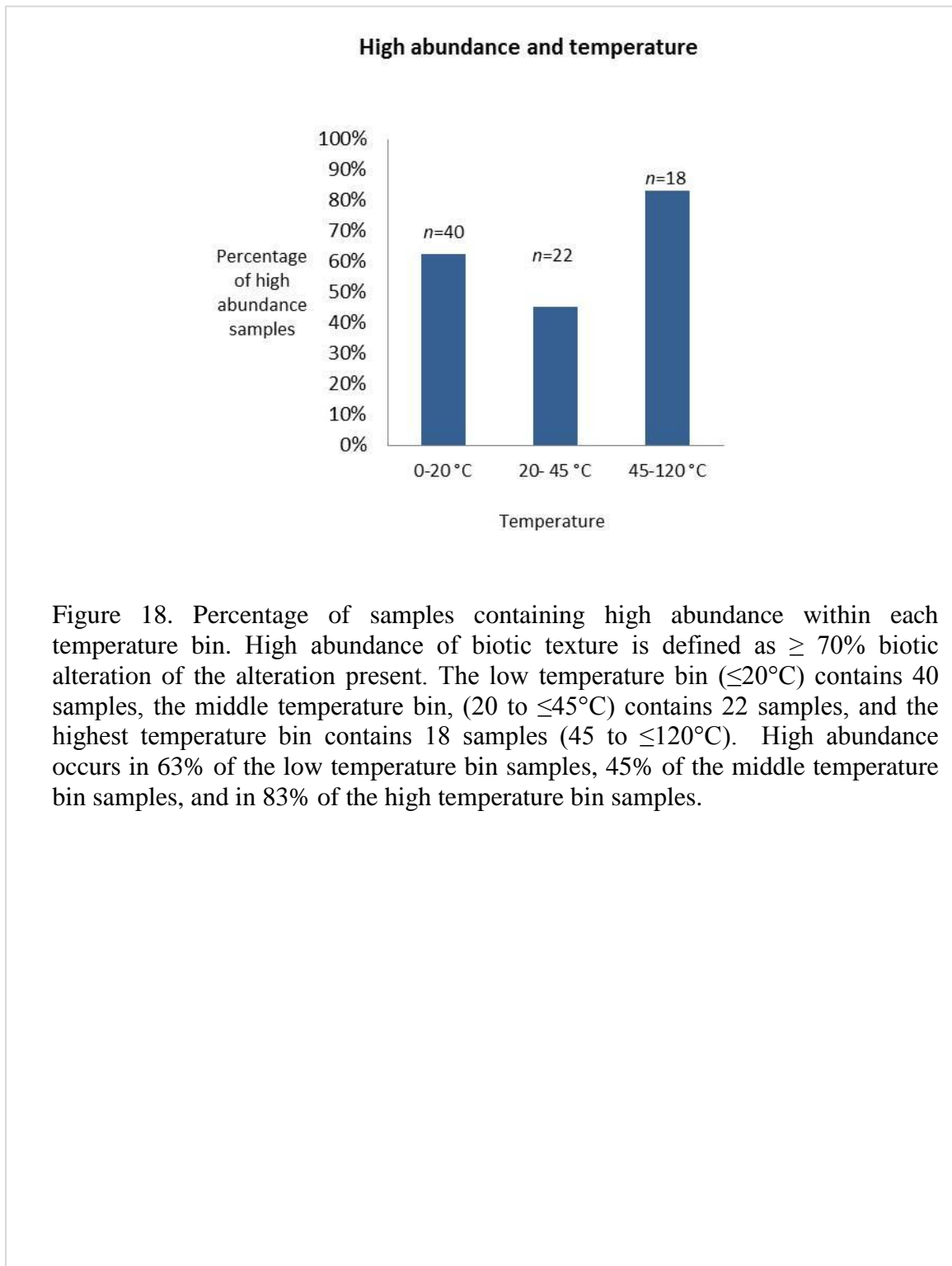
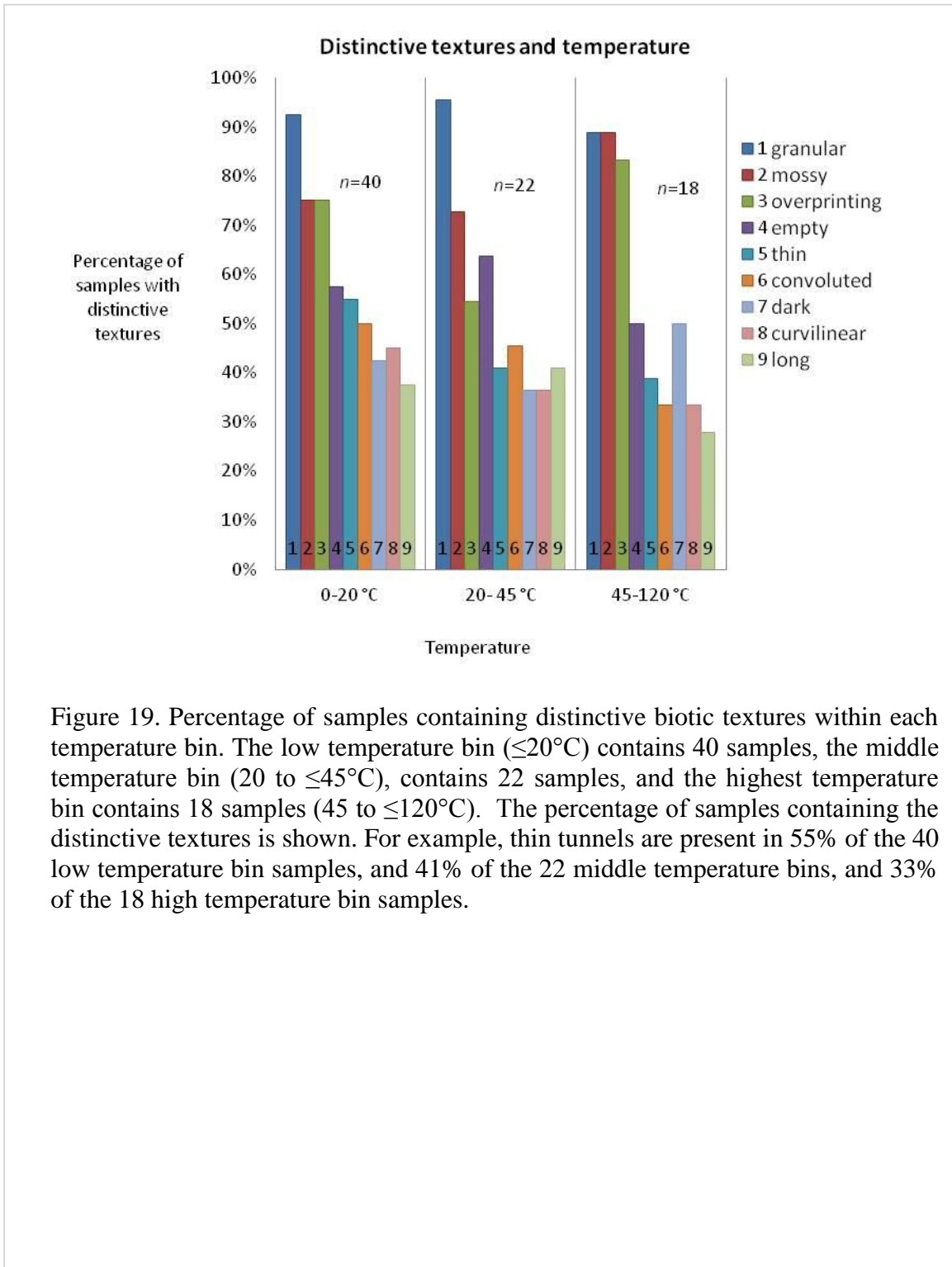


Figure 18. Percentage of samples containing high abundance within each temperature bin. High abundance of biotic texture is defined as $\geq 70\%$ biotic alteration of the alteration present. The low temperature bin ($\leq 20^{\circ}\text{C}$) contains 40 samples, the middle temperature bin, (20 to $\leq 45^{\circ}\text{C}$) contains 22 samples, and the highest temperature bin contains 18 samples (45 to $\leq 120^{\circ}\text{C}$). High abundance occurs in 63% of the low temperature bin samples, 45% of the middle temperature bin samples, and in 83% of the high temperature bin samples.



Environmental parameter V: Secondary alteration minerals

Minerals are proxies for environmental conditions (Table 2) and are used to investigate the link between biotic activity and environmental conditions (Ruffell, et al., 2001; Slate and Stevenson, 2000).

Secondary alteration minerals and abundance

High abundance of biotic alteration occurs in more than half of the samples with the respective minerals (Figure 20). Zeolites are present in 87% of the samples with a high abundance of biotic alteration. Oxidized minerals and sulfide minerals are present in 67% of the high abundance samples. Samples with carbonate minerals have high abundance of textures in 64 %, and 54% of samples with smectite minerals have high abundance. High abundance of biotic alteration occurs more frequently with zeolite minerals, than with others such as oxidized and sulfide minerals, and carbonate minerals. Zeolites typically form in a high pH environment with high salinity (Faure, 1998). Zeolites and high abundance were correlated using Spearman's correlation coefficient at the 99% confidence level ($r_s = 0.36$, $P < 0.01$) (Appendix F).

Smectite

Mica minerals and chlorite minerals are grouped here with the minerals categorized as smectite from the shipboard observations. Smectite forms under a wide range of conditions and has been synthesized under temperatures ranging from $\sim 3^\circ\text{C}$ and up to 300°C , well beyond the constraints of this study's environment (Kloprogge et al., 1999). Smectite was a frequently observed mineral and since future studies could use smectite minerals, by determining the specific ion substitutions, they are included here. Without knowing the specific mineral species such as saponite or kaolinite and the ion substitution, smectite cannot be used to make valid significant correlations in this study.

Granular, mossy, overprinting and empty tunnel textures were found in more than half the samples containing smectite (Figure 21). Granular textures were found in 91% of the samples with smectite, mossy textures in 61%, overprinting was found in 74% and

empty tunnels were found in 52% of samples with smectite. The occurrence of mossy texture and smectite in a sample together is however negatively correlated. This is confirmed with a correlation using Pearson's method ($r_p = -.23$, $p < 0.01$) (Appendix F), which is perhaps a function of a small sample size.

Carbonate

Several conditions are necessary to precipitate carbonate minerals. For example, calcite (CaCO_3) precipitation requires a high pH, low dissolved CO_2 , and high amounts of Ca^{2+} in solution (Faure, 1998). The Carbonate Compensation Depth (CCD) is a boundary determined by the physical and chemical properties of the water column. Below the CCD, CaCO_3 will dissolve because of low temperatures and high pressure. Precipitation of carbonate minerals can occur below the CCD if accumulation is greater than the dissolution rate or where the fluid is not in contact with seawater, for example where sedimentation is high, or where mineralization has reduced porosity and permeability or close to the crustal sealing age where seawater cannot circulate. The other requirements for carbonate precipitation below the CCD are low dissolved CO_2 , or CO_2 is consumed in other reactions, for example mineral precipitation, or during microbial metabolism (Konhauser, 2007).

The distinctive textures that occur most frequently in samples with carbonate are granular, mossy, overprinting and empty tunnel textures; these distinctive textures are found in more than 50% of samples with carbonate minerals (Figure 21). Granular texture is found in 93% of samples containing carbonate minerals. Of the samples with carbonate minerals mossy texture is found in 75%, overprinting is found in 69%, and empty tunnels are found in 58%. Curvilinear texture is found in 45% of samples with carbonate minerals. The presence of carbonate minerals is quantitatively correlated with mossy and curvilinear samples, more than it is correlated with the other distinctive texture types. This correlation was not confirmed with a Pearson's correlation test at neither the 95% nor the 99% confidence level.

Zeolite minerals

Zeolite minerals are commonly produced from the interaction of volcanic rocks and saline solutions, and are stable with higher pH (Faure, 1998; Lisitzina and Butuzova 1982; Chester, 1979).

The most common texture type found in samples with zeolites is overprinting, which is found in 93% of samples, granular the next highest is found in 87%, mossy is found in 67% and empty tunnels are found in 67% of samples with zeolites (Figure 21). Thin, convoluted, dark, curvilinear, and long textures are each found in less than 50% of samples containing zeolites. Overprinting occurs in the highest number of samples with zeolites, in contrast to its lower occurrence with other minerals. None of the other distinctive textures occurs most frequently in samples with zeolite minerals. Zeolite minerals are considered to have an effect on samples with overprinting in contrast to the other texture types. This correlation is substantiated with Pearson's correlation ($r_p = .24$, $p < 0.05$) (Appendix F).

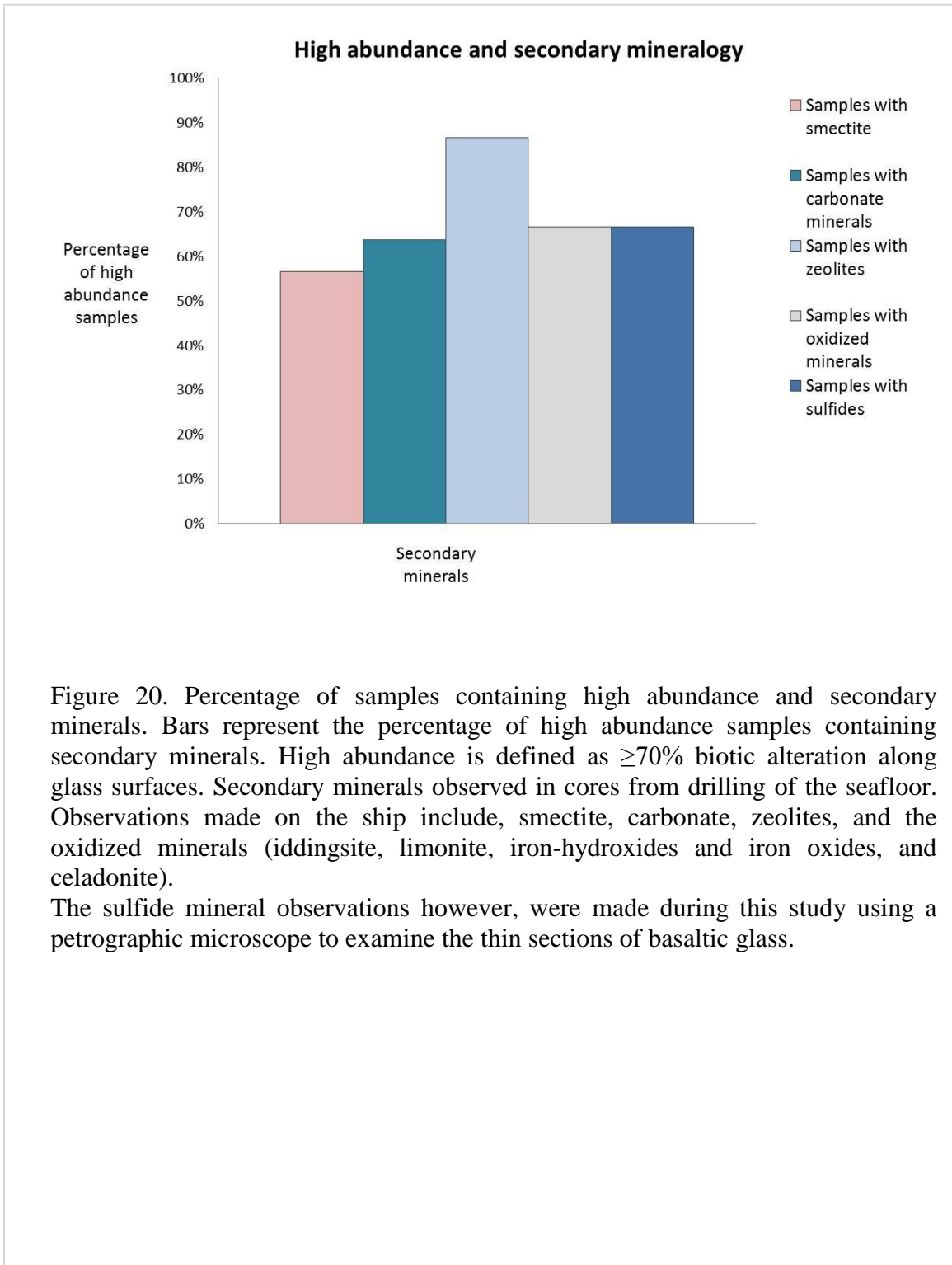
Oxidized minerals

The oxidized minerals include celadonite, limonite, iddingsite, and oxyhydroxide (Table 2). Granular, mossy, overprinting and empty textures occur in more than half the samples with oxidized minerals (Figure 21). Granular textures are found in 89% of the samples with oxidized minerals, mossy textures are found in 73%, overprinting is found in 70% and empty tunnels are found in 55% of samples with oxidized minerals. Thin, convoluted, dark, curvilinear, and long textures are each found in less than 50% of samples containing oxidized minerals. Curvilinear is the only texture that has its highest occurrence in samples with oxidized minerals. Curvilinear occurs in 45% of the samples with oxidized minerals, which is in contrast with their low frequency in conjunction with other mineral types. Oxidized minerals are quantitatively correlated with curvilinear textures more than with any other texture type. Oxidized minerals are statistically correlated with curvilinear samples ($r_p = .24$, $P < 0.05$) and long samples ($r_p = .28$, $P < 0.05$) (Appendix F). Each showed a strong statistical correlation at the 95% level.

Sulfides

The presence of sulfides could indicate microbial reduction of sulfate, or microbial metabolism of oxygen, which produces anoxic conditions, and so were investigated in this study to provide information about the environmental conditions associated with the textures. Observations of sulfides were made during the course of this study using a petrographic microscope (Appendix C).

Granular, mossy, overprinting and empty tunnels textures occur in more than half the samples with sulfides (Figure 21). Granular texture is found in 96% of samples with sulfides, mossy in 73%, overprinting in 78%, empty tunnels in 64%, and thin tunnels in 53% of samples with sulfides (Figure 21). Granular, thin, convoluted and long tunnels textures have their maximum occurrence in conjunction with sulfide minerals. Granular, thin, convoluted, and long tunnels textures are slightly more abundant in samples with sulfide minerals, than are any of the other texture types.



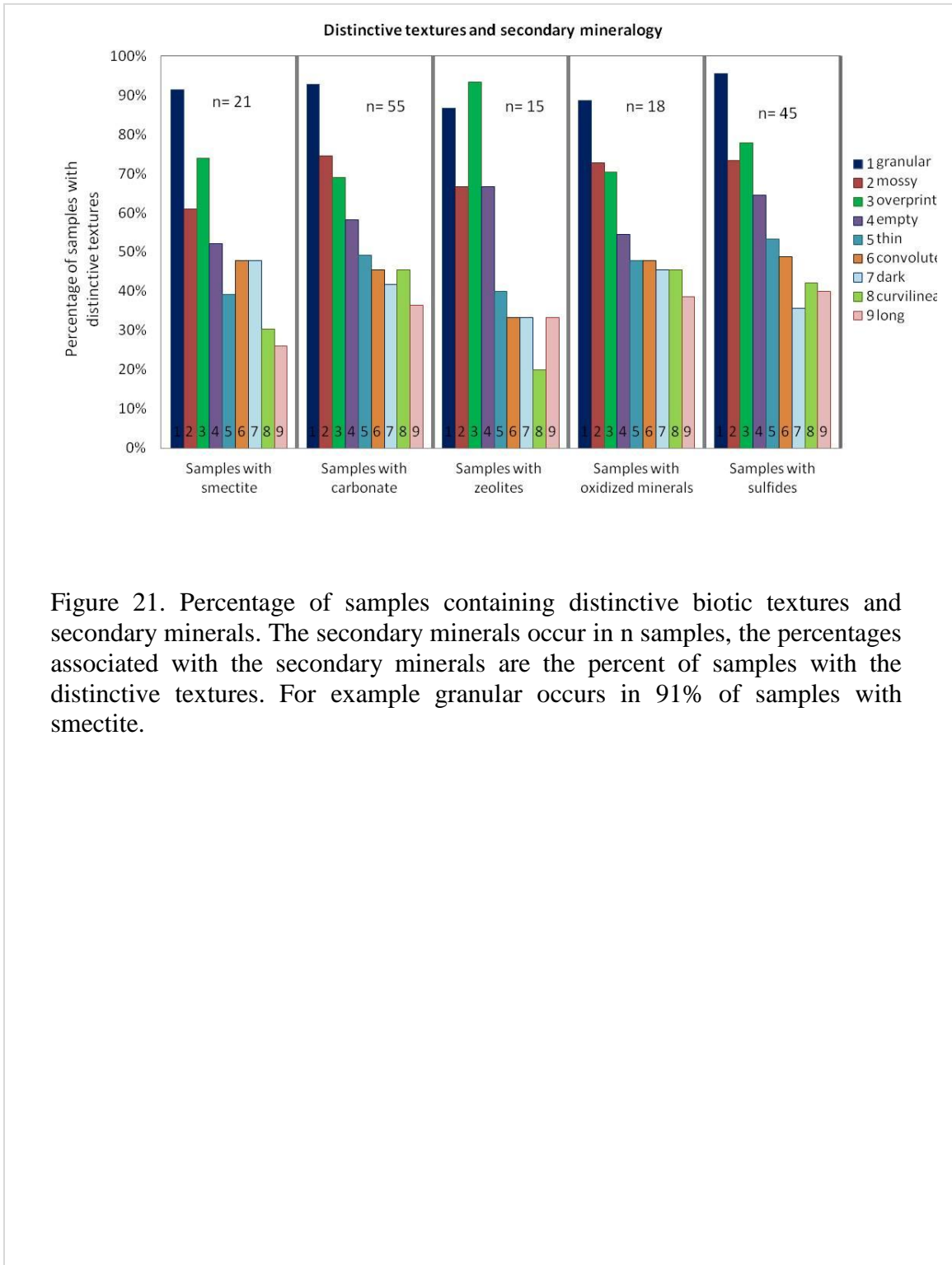


Figure 21. Percentage of samples containing distinctive biotic textures and secondary minerals. The secondary minerals occur in n samples, the percentages associated with the secondary minerals are the percent of samples with the distinctive textures. For example granular occurs in 91% of samples with smectite.

These results demonstrate that some distinctive textures and high abundance occur more frequently for certain environmental conditions (Table 5). Both the maximum and minimum percent occurrence for each texture type and high abundance with respect to the environmental parameters was recorded as an initial descriptive method of correlation as presented in Table 5.

Most distinctive textures occur in the highest number of samples in the oldest age bin. Sample depth shows similar results. Sediment thickness and temperature show varied results (Table 5). The secondary mineral show the following results: granular, thin and long tunnels have their maximum occurrence with sulfides. Overprinting, empty, and high abundance have their maximum occurrence with zeolites. Mossy and curvilinear have their maximum occurrence with carbonates. Convolute and dark tunnels have their maximum occurrence with smectite minerals (Table 5).

Table 5 Summary of the descriptive maximum and minimum trends from Figures 11-21 for the biotic alteration and the environmental parameters

		<i>I</i>			<i>II</i>		<i>III</i>		<i>IV</i>			<i>V</i>				
		Sample age			Sample depth into basalt		Sediment thickness		Temp.			Smectite	Carbonate	Zeolite	Oxi. minerals	Sulfides
Bins		0 – 20 Ma	20-65	65-160	0-50 m	50-550	30-300 m	300-1550	0-20°C	20-45	45-120					
1	granular	-	-	+	-	+	-	+		+	+			-		+
2	mossy		-	+	-	+	-	+		+	+		+			
3	overprinting	-		+	-	+	-	+		-	+		-	+		
4	empty		-	+	-	+	-	+		+	-		-	+		
5	thin	-		+	-	+	+	-	+	-	-		-			+
6	convoluted		-	+	-	+	+	-	+	-	-		+			
7	dark		+	-	+		+	-	-	+	+			-		
8	curvilinear	-	+		-	+	+	-	+	-	-		+	-		
9	long	-		+	+	-	+	-	+	-	-					+
≥70%	high abundance	-		+	-	+	-	+		-	+		-		+	
<p>+ Indicates the highest percentage of samples with distinctive textures for the environmental parameter bins are called the maximum occurrence. - Indicates the lowest percentage of samples with the distinctive textures for the environmental parameter bins are called the minimum occurrence.</p> <p>For example, the highest percentage of granular samples is found in the oldest age bin. For the graphs that the results in this table are derived from see Figures 11 through 21.</p>												<p>+ Indicates that the distinctive texture has its highest occurrence with this mineral - Indicates the distinctive texture occurs the least often with this mineral</p>				

3.3 Summary of quantitative analysis results

The correlation strength and direction between the distinctive textures and the environmental parameters was assessed using Spearman's rank correlation coefficient (r_s), and Pearson's product moment correlation coefficient (r_p) (Section 2.3.2 and Appendix F). To determine what would constitute a statistically significant relationship initial confidence levels selected before the analysis: $P < .05$ and $P < .01$. These values ensure that the null hypothesis (which states there was no correlation) would be rejected 95% and 99% of the time given the values in this study for the distinctive textures, high abundance, and environmental parameters. The values resulting from the correlation calculation (IBM SPSS Statistics v. 16.0), were compared to critical values for these confidence limits and the coefficients that fell above these limits were selected as significant (Zar, 1999). The results that demonstrate statistically significant correlations are presented in Appendix F and Table 6. This does not mean that the hypotheses $H_{1(A-C)}$ are proven true. Instead it means that the null hypotheses $H_{0(A-C)}$ are not true given these values. The null hypotheses state that there is no correlation.

The conditions listed in Table 6 in the middle column are those inferred or directly evidenced by the environmental parameters (Appendix F). If there was a significant correlation between the parameter value and a biotic alteration feature then these conditions are inferred to be relevant. The correlation coefficients, as they are calculated in this study, represent the correlation strength of two variables, e.g., long tunnels and sample age, relative to the correlation strength of the other distinctive textures, e.g., mossy tunnels and sample age (Appendix F).

The four continuous environmental parameters, sample age, sample depth into basalt, overlying sediment thickness and temperature indicated strong positive skew with respect to a normal distribution. This is evident in the scatter plots (Appendix F) of the continuous variables where majority of the samples have young sample age's, are from shallow depths, lower temperatures, and shallow overlying sediment thickness. Biotic alteration abundance showed strong negative skew because most of the abundance

estimation values are above 50% and so analyses that did not assume normality were required. For all of the four environmental parameters and biotic alteration abundance Spearman's r_s was to calculate the correlation coefficients.

Table 6 Summary of quantitative environmental parameter correlations

	<i>Conditions inferred by the environmental parameters</i>	<i>*Biotic alteration correlation result corresponding to environmental parameter</i>
<i>*High abundance of biotic alteration</i>	High abundance indicates prolific biotic activity	overprinting ($r_s = 0.24^*$)
<i>Environmental parameters</i>		
<i>I. sample age</i>	Sample age increase indicates decrease in oxygenated, nutrient-rich, fluid circulation	curvilinear ($r_s = 0.25^*$)
<i>II. sample depth into basalt</i>	Sample depth into basalt increase indicates a decrease permeability and oxygenated, nutrient-rich, fluid circulation	overprinting ($r_s = 0.29^{**}$) abundance ($r_s = 0.27^*$)
<i>III. overlying sediment thickness</i>	Overlying sediment thickness increase indicates decrease in oxygenated, nutrient-rich fluid circulation after	No significant correlation
<i>V. temperature</i>	Increased temperature indicates increased tolerance of microbial ecosystem	curvilinear ($r_s = -0.23^*$)
<i>Secondary Minerals</i>		
<i>smectite</i>	Stability conditions too broad for use as an indicator	mossy ($r_p = -0.23^*$)
<i>carbonate</i>	Stable under high pH and low CO ₂	No significant correlation
<i>zeolite</i>	Stable under high pH and high saline solutions	abundance ($r_{sF} = 0.36^{**}$) overprinting ($r_s = 0.24^{**}$)
<i>oxidized minerals</i>	Presence indicates oxidation	curvilinear ($r_p = 0.24^*$) long ($r_p = 0.28^*$)
<i>sulfides</i>	Presence indicates reduction	No significant correlation
<p>Spearman's rank correlation coefficient (r_s), Pearson's product-moment correlation coefficient (r_p). * P<0.05, **P<0.01</p> <p>*For full results see Appendix F</p> <p>**High abundance was treated as the independent variable or an environmental parameter when tested against the distinctive textures. It is considered to be a proxy for ecosystem conditions conducive to biotic activity.</p>		

4. DISCUSSION

The primary objectives of this study include determining the relationship between the biotic alteration of basaltic glass and environmental conditions. My hypothesis is that abundance, and types of biotic textures are related to the environmental parameters.

Two methods of analysis were employed, descriptive (Table 5) and quantitative (Table 6). The descriptive analysis evaluated how the distinctive textures and abundance, related to the environmental parameters. The environmental parameters were divided into groups of low, high, and intermediate values. These divisions were made to reflect characteristics of the crust, especially the seafloor conditions and the ecosystem conditions (Table 4). The maximum occurrence (Table 5) for each texture type, and high abundance, was investigated to see if either corresponded with a particular environmental parameter value range (Figure 12 through Figure 21). This provided insight into how texture types and abundance related to the environmental parameters.

The quantitative method of data analysis used correlation coefficients to gauge the strength and direction of correlation (Table 6 and Appendix F). The alternative hypotheses tested (Section 2.3.2) were that biotic alteration texture type and abundance varied with environment and the null hypotheses tested were that there were no correlations present. In this section, the results of these two methods are discussed in terms of the environmental parameters and their relationship to biotic alteration.

In determining whether correlations exist, the statistically significant correlations are considered the most robust indicator of relationships (Table 6 and Appendix F). The statistical results are compared with the binned descriptive analyses (Figures 10 through 21 and Table 5). It is important to note that the statistical correlations were conducted using continuous data for the parameters, sample age, depth into basalt, overlying sediment thickness, and temperature (Appendix C), so a direct comparison with the descriptive method is inappropriate. A maximum occurrence of a distinctive texture or

high abundance with respect to a bin is considered a descriptive representation of the statistical relationship.

4.1 Biotic alteration

The descriptive method of investigation defines abundance as the percentage of alteration that is produced by biotic forces within the rock (Figure 6, Figure 10 and Appendix C). The second part of investigating biotic alteration, includes biotic texture type classification, was based on the morphology and size of the textures that comprise the biotic alteration (Figures 7 and Figure 8).

For the initial descriptive examination, the abundance of biotic alteration was divided into two categories, high abundance >70% and low abundance <70% (Figure 6 and Figure 10). For the correlation calculations, Pearson's (r_p), the abundance results were left continuous to increase the population size for consideration ($n=80$) (Table 6 and Appendix F). Nine distinctive textures were selected as the most commonly occurring texture types, (1) granular, (2) mossy, (3) overprinting, (4) empty, (5) thin, (6) convoluted, (7) dark, (8) curvilinear, and (9) long (Figures 7,8, and 9).

4.1.1 Biotic alteration abundance

Biotic alteration is common in all samples (Table 3 and Figure 10). More than half the samples were classified as containing high abundance (high abundance indicates 70% or more biotic alteration) (Figure 10). The average biotic abundance value for all samples was 70%. There were no samples with 0 or 10% abundance of biotic alteration and so the lowest percentage of biotic alteration abundance observed was 20%. For example, 16 samples contain 100% biotic alteration, 10 samples contained 50%, 7 samples contain 40%. High biotic alteration is therefore more common than low abundance. This is consistent with what Furnes and Staudigel (1999) found. They found that in the upper 250 meters abundance values of 65 % to 80% abundance were the most common. Cousins et al., (2009) also found that for dominantly marine samples, values of >60% abundance was the most common.

4.1.2 Biotic textures and distinctive biotic textures

Although in some cases only one or two texture types were present in a sample; within a discrete area, such as a fracture, or around a variole, three to five biotic textures were sometimes observed (Appendix A). Possible explanations of why the types of biotic textures vary in this way are: the microbes producing all the textures are diverse and the textures reflect this diversity by varying in distribution. Sometimes only one texture is observed and sometimes multiple textures in one discrete area of a sample is observed. Alternatively, there is a limited diversity and only a few or one species of microbe produces a range of texture morphologies and sizes.

The most common texture types are granular, overprinting, mossy, empty, thin, convoluted, curvilinear, dark, and long, all of which occur in over 30% of the samples or, at least 29 out of the 80 samples (Figure 9). Either, these texture types represent dominant groups of microbes, or perhaps one microbe type that is ubiquitous and produces all these texture types. The least common texture types are separate, simple, and engorged, each occurring in only 2 of the 80 samples (3%). The wide diversity of microbes found living in communities on seafloor basaltic lava surfaces suggests that oceanic basalts could host a more diverse community of microbes than the overlying seawater (Santelli, et al., 2008). The presence of multiple texture types along one habitable region, such as a fracture, or variole could be a reflection of this diversity.

4.1.3 Biotic textures and abundance

The most common high abundance biotic textures were, granular, mossy, and overprinting (Figure 11). Overprinting was the only texture type to show a statistically significant correlation with increasing abundance ($r_s = 0.24$, $P < 0.05$) (Table 6 and Appendix F). This is not surprising because overprinting is by definition the superposition of alteration fronts, indicating multiple episodes of alteration. Therefore, the amount of alteration textures expected would be higher than in a sample with only one alteration episode.

4.2 Biotic alteration and environmental parameters

4.2.1 Environmental parameter I. Sample age

For the descriptive results, the samples were sorted into bins, young (0-20 Ma), middle (20-65 Ma), and old (65-160 Ma), (Table 4), to account for stages of alteration, oxic seawater-basalt interaction, an intermediate phase, and the late stage anoxic conditions with waning fluid circulation. For the correlation analysis, Spearman's (r_s) was calculated and the age data was left continuous to maintain a maximum population size ($n=80$) (Table 6 and Appendix F).

High abundance and sample age

High abundance of biotic textures is found in samples of all age groups (Figure 12). By 20 Ma, over half the samples contain biotic alteration suggesting biotic abundance begins relatively early and continues through time since the oldest age bin >65 Ma contains the highest abundance of biotic alteration (Figure 12). In a study conducted by Furnes et al. (2001a) the samples, divided into 0-6 Ma, 10-32 Ma, and 110 Ma, were found to contain the same quantity of biotic alteration. Possible explanations proposed by Furnes et al. (2001a) were that biotic alteration occurs early, or that biotic alteration requires a long time period. The results in this thesis are consistent with biotic alteration occurring early and also with the process requiring a long time since abundance was highest in the oldest age bin, >65 Ma, and was established in over half the samples by 20 Ma. Both the proposals made by Furnes et al. (2001a) are therefore verified by the results of this thesis.

Biotic alteration is a progressive process and higher abundance could reflect later stages of alteration. Even though high abundance of biotic alteration is common in older samples, there was not a statistically significant correlation between sample age and high abundance (Table 6 and Appendix F).

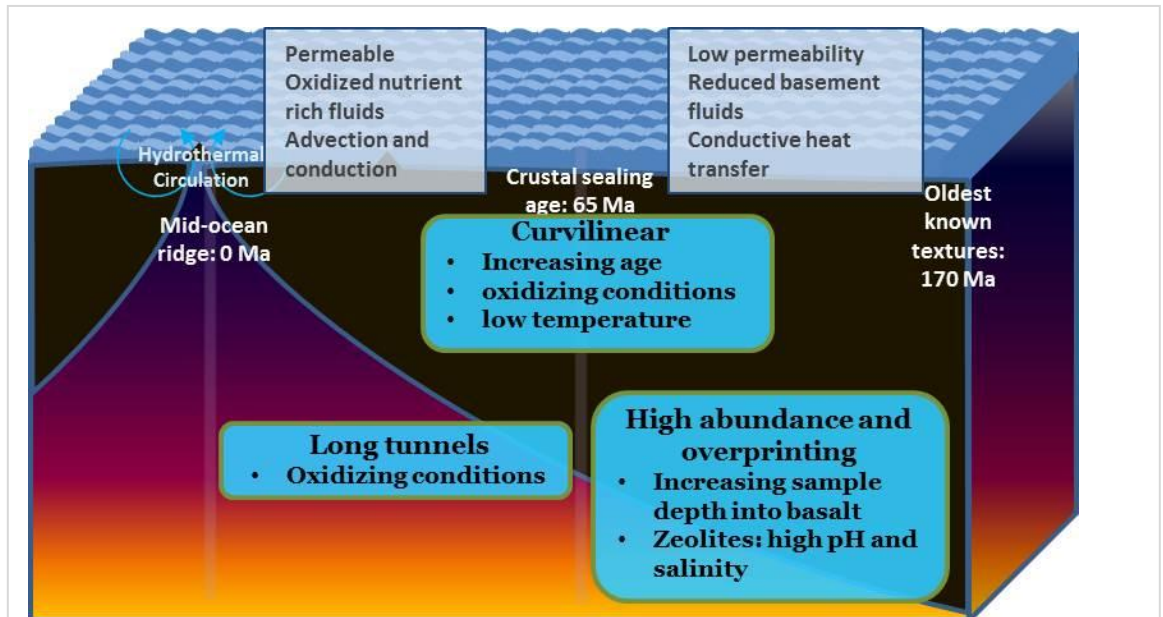


Figure 22. Schematic summary of correlations between biotic alteration and the environmental parameters in terms of oceanic crustal evolution through time.

This diagram shows a cross-section through the basaltic layer (2a) of the oceanic crust. As the crust ages the influx of nutrient rich oxygenated seawater decreases and the basement fluids become increasingly reduced. Prolonged water-rock interactions raise the fluid pH and saline content.

Curvilinear and long tunnels, overprinting, and high abundance of biotic alteration showed significant correlations with certain environmental parameters (Table 6). Specifically, curvilinear tunnels are correlated with increasing age, oxidizing conditions (as indicated by oxidized minerals), and low temperatures. Because temperature increases with age and they are correlated with increasing age, curvilinear tunnels is positioned at the mid-point of the intermediate phase of crustal evolution (~65 Ma). Long tunnels are correlated with oxidizing conditions and so are associated with the oxygenated seawater circulation present from 0 to 65 Ma. High abundance and overprinting are correlated with increasing depth into basalt and prolonged water-rock interactions as indicated by the correlation with zeolites and so both are positioned at the latter stages of crustal evolution (>65 Ma), deeper in the crust. The oldest known sample containing textures is 170 Ma.

Modified from Edwards et al., 2012; Fisher and Becker, 2000; Becker and Fisher 2000; Orcutt et al., 2011; Stein and Stein, 1994).

Distinctive textures and sample age

No texture type occurs more frequently in the youngest age group relative to the other age groups, although most texture types were present in at least 20% of the samples in the younger bin (Figure 13). This distribution suggests that texture formation for all texture types begins in young crust. In the middle age bin, dark tunnels and curvilinear tunnels occur in the highest frequency, in contrast to their lower occurrences in other age ranges. The other textures, granular, overprinting, empty, thin, convoluted, and long, exhibit a maximum occurrence in the oldest sample range. This suggests that perhaps these textures form during all periods, and by the oldest crustal ages they can be found in many locations. The oldest sample containing textures found to date is 170 Ma old (Fisk et al., 1999).

The one texture type with a significant correlation was curvilinear which showed a correlation with increasing sample age ($r_s = 0.25$, $P < 0.5$) (Table 6 and Appendix F). A study by Furnes et al. (2001a) found that the proportion of biotic alteration to abiotic alteration did not change with an increase in sample age. This is consistent with the predominant lack of correlation found here with the exception of the curvilinear negative correlation. Biotic alteration likely begins early in the evolution of the crust (Furnes et al., 2001a).

4.2.2 Environmental parameter II. Sample depth into basalt

For the descriptive results sample depth into basalt was divided into two categories, shallow (0-50 m) and deep (50-550 m) to account for higher permeability at shallower depths (Table 4). For the correlation analysis, Spearman's correlation coefficient (r_s) was calculated the sample depths data were left continuous (Appendix C) to maintain a maximum population size ($n=80$) (Table 6 and Appendix F).

High abundance and sample depth into basalt

High abundance is found in half the samples from shallower than 50 meters depth and increases to 74% with increasing depth suggesting that increasing depth has a

positive influence on the abundance of biotic activity, and also that high permeability is not required for biotic alteration (Figure 14). In this study Pearson's correlation, which was calculated with continuous data indicates a significant positive correlation between high abundance and increasing depth ($r_s = 0.27$) (Figure 22, Table 6, and Appendix F).

In a study conducted by Furnes and Staudigel (1999) at depths less than 50 meters abundance ranged from about 60% to 90% for the Atlantic Ocean, and from about 50% to 90% for the Pacific Ocean. The fact that biotic alteration is observed in amounts >50% is consistent with the finding in this thesis that biotic alteration is commonly found to be at least 50% of the total alteration. There is a disparity between the research in this thesis that suggests abundance increases with depth and an observed decrease in abundance with depth in the Furnes and Staudigel (1999) study. This could be due to several reasons. First, a direct comparison might not be appropriate because their study dealt with multiple sample measurements from four locations. This thesis research observes an overall increase in abundance with depth from many locations. In a later study, Furnes et al., 2001a found a decrease in abundance for a larger sample set than the four locations. In the Furnes et al. (2001a) study the limitations of seafloor sampling were enumerated and could provide insight into the differences between the study of multiple samples from one hole and one sample for many holes. These include: low recovery rates of material, <50%, for a conservative estimate, and the potential for fragile glass biotic textures to be destroyed in this environment and even during drilling. A possible way to overcome this is the use of multiple samples. The ideal method is to have multiple samples from many locations, a combination of the Furnes and Staudigel (1999) and the Furnes et al. (2001a) method and the method used in this thesis study could overcome these limitations. The question remains however, whether permeability is a predominant influence on biotic activity in the ocean crust.

Distinctive textures and sample depth into basalt

Because all texture types occur more frequently in deeper samples than in shallower samples, biotic activity is perhaps a process that occurs at all depths (Figure 15). Other

explanations could be revealed by more samples from the locations observed in this study. Overprinting was found to be statistically correlated with increasing sample depth into basalt ($r_s = .24$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). Because overprinting indicates multiple alteration fronts this corroborates the tendency of abundance to increase with depth.

4.2.3 Environmental parameter III. Overlying sediment thickness

Sediment thickness was divided into two categories, moderate sediment (30-300 m), and thick sediment (300-1550 m), in order to distribute the samples evenly (Figure 24, Table 3, and Table 4) and because several hundred meters of sediment is proposed to hydrologically isolate the igneous basement allowing water to enter only from distant outcrops and not locally (Stein and Stein, 1994).

High abundance and overlying sediment thickness

Of the three large scale variables, age, depth into basalt, and overlying sediment thickness, overlying sediment thickness shows the least correlation with both distinctive texture types and high abundance (Figure 16). Biotic abundance is approximately equal for thin and thick sediment values. Changes in sediment thickness do not correlate with changes in the abundance of biotic alteration (Table 5). For the correlation analysis, Spearman's (r_s) was calculated and the overlying sediment thickness data was left continuous to maintain a maximum population size ($n=80$) (Appendix F).

Distinctive textures and overlying sediment thickness

Both mossy and convoluted tunnel textures show an insignificant increase in abundance with depth (Figure 17). The individual texture types show no tendency to occur preferentially in areas with thick or with thin overlying sediment. Overall, the depth of sediment has no significant influence on distinctive texture type or biotic abundance for the samples examined.

That biotic activity is not influenced by overlying sediment thickness indicates that deep sediment, which creates a hydrologically isolated igneous basement in terms of influx of seawater, does not drive texture development.

A study by Furnes et al., 2001a found that the proportion of biotic alteration to abiotic alteration was unresponsive to an increase of sediment thickness which corroborates the results here. Because it may be a proxy for influx of oxygenated seawater, this suggests the microbial communities are unresponsive to a lack of oxygenated seawater circulation.

4.2.4 Environmental parameter IV. Temperature

Temperature was divided into three bins based on optimum growth ranges for microbes, psychrophile (0-20°C), mesophile (20-45°C), and thermophile and hyperthermophile (45-120°C) (Table 4). For the correlation analysis, Spearman's (r_s) was calculated and the temperature data was left continuous to maintain a maximum population size (n=80) (Appendix F).

High abundance and temperature

The presence of more high abundance samples in the thermophile and hyperthermophile temperature bin, suggests that the biotic activity responds positively to an increase in temperature (Figure 18). Higher temperature environments therefore are more effectively colonized leaving a higher quantity of biotic alteration as evidence in the rocks. Alternatively, the biotic alteration occurs at lower temperature and the evidence simply remains after the temperature of the rock is increased due to burial and crustal sealing.

Distinctive textures and temperature

The presence of the distinctive textures varied with temperature, which suggests that the microbial systems instrumental to the creation of texture morphology are better adapted to certain temperature ranges than others (Figure 19). Thin convoluted, and curvilinear occur more frequently in colder samples; granular, empty, and long occur

more frequently in the mesophile temperature range; and mossy, overprinting and dark occur more frequently in the higher temperatures.

If the tendency towards higher temperatures is valid and there is a limit on the range of temperatures at which a particular texture type formed, this would be evident in the samples. For example, a sample that was originally cold would contain thin, convoluted, and curvilinear tunnels, but they would be overprinted by the “higher temperature textures”. If a sample did not undergo burial or some other temperature changing process, its limited temperature exposure would be visible by the lack of diversity in the texture types. The various textures that produce an overprinted texture are often hard to identify. It was still possible however, to observe that the overprinting of the “lower temperature textures” indicated in this study, thin, convoluted, and curvilinear, were not overprinted by mossy, or dark, the “higher temperature textures” on a consistent basis, short tunnels, wide tunnels, network tunnels and others are also commonly overprinted.

The only statistically significant correlation was for curvilinear texture ($r_s = .23$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). Overall temperature is not a conclusive ecosystem control which is consistent with the findings of Furnes et al., 2001a.

4.2.5 Environmental Parameter IV. Secondary mineralogy

Shipboard observations of drill cores included the alteration mineralogy that was visible in hand sample size pieces (Table 2 and Appendix C). Pearson’s product moment correlation coefficient was calculated at the 95% and the 99% confidence levels (Appendix F).

Smectite

Smectite was not a strong indicator of environmental conditions because it is stable under a large range of conditions (Faure, 1998). No particular distinctive textures or high abundance was correlated with smectite (Figure 21). Mossy texture was found to have minimum occurrence in the presence of smectite (Table 5). The presence of mossy texture and smectite together in a sample is negatively correlated ($r_p = -0.23$, < 0.05)

(Table 6 and Appendix F). Since smectite is stable under a wide range of conditions, including temperature, oxidation state and pH (Faure, 1998), an explanation of the negative correlation between mossy texture and smectite is not indicative of any ecosystem conditions. This could be an artifact of a small sample size relative to the global environment or some variable not tested here.

Carbonate

The distinctive textures and biotic abundance were found to show very little correlation with the presence of carbonate (Figure 21 and Table 6). Because carbonate minerals are an indicator of low dissolved CO₂ and higher pH, perhaps high pH is conducive to biotic activity.

Zeolites

Overprinting ($r_p = 0.24$, $P < 0.05$) and high abundance ($r_s = 0.36$, $P < 0.05$) were correlated with the presence of zeolites (Figure 22, Table 6 and Appendix F). Zeolites are found on average to have only a minor correlation with texture type, but there is a correlation between zeolites and increasing abundance. The correlation between high abundance of biotic textures and the presence of zeolite minerals suggests that perhaps high pH is an environmental condition conducive to the proliferation of these microbial systems. As the crust ages, prolonged water rock exposure increases salinity. This is consistent with a correlation between overprinting, and high abundance (Figure 22, Table 6, and Appendix F).

Pyrite

The presence of pyrite was determined by sample observations aboard ship. Therefore the pyrite is a macroscopic mineral that was probably deposited in veins as a late secondary phase and for this reason is not associated with the initial alteration of the basaltic glass. Pyrite was only observed in five samples of the 80 samples. Because of this, it is unlikely there is a correlation with pyrite and biotic alteration.

Oxidized minerals

Texture formation, or biotic texture proliferation is not significantly correlated with oxidized minerals and none of the distinctive textures show a maximum occurrence with oxidized minerals (Figure 21 and Table 5). High abundance of biotic alteration is not quantitatively correlated with oxidized minerals (Table 6 and Appendix F). This is consistent with the persistence of high abundance samples and a greater occurrence of distinctive textures in deeper crust, and therefore with more reduced fluids. These results suggest that the microbes that produce textures have a tendency towards reducing conditions. There was however, a statistical correlation between oxidized minerals and biotic alteration (Appendix F). Oxidized minerals are quantitatively correlated with curvilinear tunnels ($r_p = 0.24$, $P < 0.05$) and long samples ($r_p = 0.28$, $P < 0.05$). Each showed a strong statistical correlation at the 95% level (Figure 22, Table 6, and Appendix F).

Sulfide minerals

Microscopic sulfide minerals observed with a microscope in the samples are not strongly quantitatively correlated with high abundance (Table 6 and Appendix F). Granular texture, thin tunnels, and long tunnels have maximum occurrences with sulfide minerals, perhaps suggesting a relationship between granular texture, thin and long tunnels and reducing conditions.

4.3 Speculation of biogenetic conditions from descriptive analysis

4.3.1 Granular

Granular texture is established in the first 20 Ma and is found in most samples in all the bins, for sample age, basalt depth, temperature, and sediment thickness (Figures 13, 15, 17, and 19). Granular texture is evenly distributed with respect to the secondary minerals, and occurs in high percentages for all of them (Figure 21). This suggests granular texture is formed under most conditions and is not correlated with any particular environmental parameter in this study.

Granular texture is made up of small spheres instead of tunnels. The size and shape of the spheres have caused some authors to suggest that they are created around microbial cells of similar size and shape, which are found near the granular texture (Thorseth et al., 2001). An alternative hypothesis is that the granular texture is not biotic. Evidence supporting this possibility is that granular texture differs from the morphological complexity of the tunnels (Figure 4 and Figure 7). Also, granular texture is present in 93% of all the samples (Figure 9), and no particular environmental conditions are correlated with granular texture, which suggests that it can form under a wide variety of conditions.

Granular texture resembles etch pits formed by microbes (Thorseth et al., 1992) and inorganic acids in the laboratory Fisk et al. (2013). In both studies, the proposed mechanism that would produce the etch marks on the glass were acids in the environment. Since microbes such as fungi (McLoughlin et al., 2010b) produce organic acids granular texture could be a passive indicator of biotic activity resulting from ambient acidic conditions (Thorseth et al., 1992). Alternatively, since inorganic acid produced the etch pits in the study by Fisk et al. (2013) perhaps granular texture is not related to biotic activity at all.

4.3.2 Mossy

Mossy texture is established in the first 20 Ma, and occurs at greater frequency in older samples, and in samples from greater basalt depths, thicker sediment, and higher temperatures (Figures 13, 15, 17, and 19). The high occurrence of mossy texture measured with all conditions suggests that it was produced in a wide range of environments. Mossy therefore could be formed by microbes capable of surviving in both oxic conditions and anoxic conditions, and over a range of temperatures, basalt depths and sediment thicknesses. Its negative correlation ($r_p = -0.23$, $P < 0.05$) with the presence of smectite is difficult to interpret because of the wide range of temperature, pressure and pH conditions smectite is stable under.

4.3.3 Overprinting

Overprinting is established in the first 20 Ma (Figure 12), but it occurs most often in older, deeper samples ($r_s = 0.29$, $P < 0.99$) (Figure 22, Table 6, and Appendix F) indicating that overprinting occurs progressively over time. This correlation and the correlation with high abundance ($r_s = 0.24$, $P < 0.05$) (Appendix F) is consistent with multiple stages of alteration. An affinity with high pH is indicated by the statistically significant correlation with zeolite ($r_p = 0.24$, $P < 0.05$). High pH is consistent with the progressive formation of phyllosilicates and carbonate will increase pH over time by removing CO_2 (Faure, 1998). It remains unknown however if the overprinting process occurs continuously, or in discrete alteration periods. Perhaps this is a species dependent process, e.g., a more common microbe that is readily introduced, or adapted to a broader range of conditions.

4.3.4 Empty tunnels

Empty tunnels are present in samples from the first 20 Ma, but they occur in more samples in older age bins (Figure 12). There is little change in the percentage of its occurrence with depth or sediment thickness, although empty tunnels decrease in the highest temperature range. This suggests that empty tunnels form primarily early in the crustal evolution process. The examination of secondary mineralogy shows that empty tunnels are not correlated with samples containing zeolites, and sulfides, which are all late stage alteration minerals suggesting that empty tunnels form predominantly early in the aging process.

Empty tunnels could represent the earliest biotic alteration. In older samples they are perhaps overprinted or filled in. Empty tunnels occur most frequently with mesophilic growth range (Figure 19). Since burial can increase the temperature of a sample, empty tunnels could form during the first 65 Ma when fluids are still circulating and the temperature could increase after the tunnels form. This is consistent with the presence of empty tunnels in all age groups.

4.3.5 Thin tunnels

Thin tunnels occur in a higher percentage of samples in the higher age bins (Figure 12). They occur in less than half the samples younger than 20-65 Ma age range. Thin tunnels are distributed fairly evenly across depth ranges and sediment thicknesses, and are found to decrease in occurrence in the higher temperature ranges. This suggests thin tunnels form predominately in the intermediate evolutionary stages of crustal evolution close to, but prior to sealing age, because they are established pre 65 Ma.

4.3.6 Convoluted tunnels

Convoluted tunnels' frequency of occurrence does not change with age or sediment thickness (Figure 13 and Figure 17). They occur most often at greater depths (Figure 15), and less often with higher temperatures (Figure 19). Of all the secondary minerals zeolites occur most frequently in the presence of convoluted tunnels (Figure 21 and Table 5). Convoluted tunnels occur most often at psychrophilic growth ranges and the occurrence of convoluted tunnels decreases with increasing temperatures (Figure 19).

4.3.7 Dark tunnels

Dark tunnels decrease in frequency with increasing age and temperature. Their occurrence does not change in frequency with depth or age. They occur most frequently with zeolites. Their decrease with age could be related to the destruction, or remineralization of the dark material in the tunnels.

The presence of dark tunnels is correlated with microscopic sulfides suggesting a relationship with microscopic reducing conditions. They decrease in older samples, which could indicate a short window of opportunity when dark tunnels are produced. Dark tunnels occur most often at mesophilic, thermophilic, or hyperthermiphilic microbial environments.

4.3.8 Curvilinear tunnels

Curvilinear tunnels occur most often with increasing age bins, and are correlated with increasing age in the continuous analyses using Spearman's coefficient. They are the only texture type whose presence is negatively correlated with temperature. ($r_s = -0.23$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). Curvilinear tunnels are also strongly correlated with zeolites. Their maximum occurrence with old samples, and zeolite, suggest that curvilinear tunnels form in late stage crustal development.

Curvilinear tunnels are correlated with increasing age, which suggests that they form in older crust ($r_s = 0.25$, $P < 0.05$). This could mean that curvilinear tunnels only form in older crust at lower temperatures under anoxic conditions, or in late stage oxic conditions. Lower temperatures suggest psychrophilic or mesophilic microbes as evidenced by a negative correlation with warmer temperatures ($r_s = -0.23$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). This is contradictory to the positive significant correlation with oxidized minerals ($r_s = .24$, $P < 0.05$).

4.3.9 Long tunnels

Long tunnels occur most frequently in the samples in the oldest age bin (Figure 13). They are found in only 25% of all samples in the first 20 Ma. Their presence is not associated with sample depth into basalt (Figure 15), or with sediment thickness (Figure 17). They decrease in occurrence with high temperatures (Figure 19). Their highest percentage occurs in samples with smectite minerals. Long tunnels are significantly correlated with oxidized minerals ($r_p = 0.28$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). These findings may indicate that oxidizing conditions are favorable for long tunnel formation.

4.3.10 High abundance of biotic alteration

High abundances (> 70% fracture and linear surface with biotic alteration) are well established in the first 20 Ma (Figure 12), and are found in a higher percentage of samples from deeper in the crust (Figure 14). Similarly, high temperatures are most often

found associated with higher abundances (Figure 18) suggesting that biotic alteration begins early and continues throughout the crustal alteration process. High abundance is correlated with overprinting (Figure 11), which suggests that overprinting is a sign of prolific biotic activity ($r_s = 0.24$, $P < 0.05$) (Figure 22, Table 6, and Appendix F).

5. CONCLUSION

5.1 Biotic alteration

Biotic alteration is a common phenomenon in basaltic glass in the oceanic crust and it was observed wherever altered volcanic glass occurred (Figure 10). It can be distinguished from abiotic alteration based on complexity and irregularity of the alteration fronts, the lack of symmetry around fractures, crystals, vesicles, and varioles, and the shape and size variation. The amount of biotic alteration and the type of biotic alteration can be estimated (Furnes et al., 2001a) (Figures 6 and Figure 10 and Appendix C, this study).

5.1.1 Biotic alteration abundance

High abundance of biotic alteration occurs in more samples than low abundance does (Figure 10). Also the amount of biotic alteration is measured as a percent of the total alteration along glass surfaces (Figure 6) so the high percentages of biotic abundance indicates that biotic abundance is more common than abiotic alteration (Figure 10). Biotic alteration abundance is correlated with overprinting ($r_s = .24$, $P < 0.05$) (Figure 22 and Table 6). This indicates that overprinting represents prolific biotic alteration which is consistent with the definition of overprinting that it is a multiple alteration phases.

5.1.2 Biotic textures and distinctive biotic textures

Biotic textures are a common feature in subseafloor basaltic glass (Appendix A). The most common texture type was granular (Figure 9). Because granular was found to occur in high amounts in all environments (Figures 13, 15, 17, 19, and 21) the question is raised whether granular is in fact produced by biotic process. The idea that granular is possibly not biotic is corroborated by a study conducted by Fisk et al., (2013) where granular like circular features were created in a laboratory environment using hydrofluoric acid. The experimental conditions were analogous to naturally occurring low pH environments. In this study granular texture occurs under a wide range of environmental conditions and

was found in almost all of the samples in this study (93%). Lastly, granular texture is far less distinct than the complex tunnel morphologies, and therefore is also less reminiscent of microbial forms, or microfossils.

The biotic textures are not homogeneously distributed and instead some occur more frequently than others (Figure 9). This study sought potential environmental drivers for the frequency, and distribution, variation of the distinctive textures. An explanation for why some texture types occur in a higher percentage of samples than others was not clearly established in this study. The varied distribution alone does indicate something is controlling the texture and if they are biotic than it is very likely it is an environmental condition within habitable range. The unexplained distribution and frequency observed in this study does support the conclusion that more information about the environment than what is provided here is necessary for understanding the mechanism by which they form. The data from the DSDP and the ODP are not effective for defining fluid chemistry, measured *in situ* temperature, and flow rates in the aquifer and these could be used as starting points of future investigations.

5.2 Environmental parameters

5.2.1 Environmental parameter I. Sample age

Some distinctive textures preferentially occur based on differing sample ages. However, most texture types are found in seafloor of all ages with a few types well established in young crust. It can be concluded that time is required for the textures to form. This is evident in the increase in texture occurrences in the older samples (Figure 13). The one texture type with a significant correlation was curvilinear (Table 5) with increasing sample age ($r_s=.25$, $P<0.5$) (Figure 22, Table 6, and Appendix F). Sample age does not correlate with biotic alteration abundance (Table 6 and Appendix F). This suggests the relationship between the potential microbial community and sample age, oxidizing conditions, is not very large. Furnes et al. (2001a) also found that the proportion of biotic alteration to abiotic alteration was unresponsive to an increase in

sample age. Therefore biotic alteration begins early in the evolution of the crust (Furnes et al., 2001a). The nature of how biotic alteration of volcanic glass progresses over time however remains elusive.

5.2.2 Environmental parameter II. Sample depth into basalt

All textures except dark tunnels occur in a higher percentage of deep samples than shallow samples suggesting the permeability and fluid flow are not controlling factors of microbial proliferation in terms of most texture types (Figure 15). Statistically significant correlations were found between overprinting ($r_s = .29$, $P < 0.01$) and high abundance of biotic alteration ($r_s = .27$, $P < 0.05$) with increasing sample depth into basalt (Figure 22, Table 6, and Appendix F). Sample depth into basalt is found to have minor association with biotic alteration. In studies conducted by Furnes and Staudigel (1999) and Furnes et al., (2001a) the limitations of seafloor sampling were enumerated which could have a larger effect on sample depth into basalt investigations than the other environmental parameters. These include low recovery rates of material, the potential for glass to be destroyed during sampling and over time, and the destruction of fine scale features such as these textures. These limitations suggest more data is required to resolve the relationship between sample depth and biotic alteration.

5.2.3 Environmental parameter III. Sediment thickness

Sediment thickness is not quantitatively correlated strongly with any texture type or abundance suggesting that the tendency of sediment to hydrologically isolate the basement does not correlate with changes in biotic activity and that perhaps an imperviousness to aging basement fluids exists (Figures 16, 17, and Table 6). Furnes et al. (2001a) found that the proportion of biotic alteration to abiotic alteration was unrelated to sediment thickness which corroborates the results here: overlying sediment thickness is not an important control. Because sediment thickness may be a proxy for influx of oxygenated seawater, this suggests the microbial communities are not affected by a lack of oxygenated seawater circulation.

5.2.4 Environmental parameter IV. Temperature

There was a statistically significant negative correlation between temperature and curvilinear samples ($r_s = -.23$, $P < 0.05$) (Figure 22, Table 6, and Appendix F). Because this was the only significant correlation, perhaps curvilinear could be formed at lower temperatures prior to the sample heating. Without direct temperature measurements it is difficult to resolve the timing of the relationship between texture formation and lower temperatures at the sediment basement interface. Future work could be to culture microbes adapted to specific temperature ranges to observe an effect they would have on the glass. Measured *in situ* down hole temperature measurements as opposed to the modeling done here would be beneficial in understanding temperature as an ecosystem control. This lack of conclusive results is consistent with a study conducted by Furnes et al. (2001a) where it was found that temperature fluctuations in the crust are not adequately known to produce conclusive results.

5.2.5 Environmental parameter V. Secondary mineralogy

Smectite is not useful in the correlation of biotic alteration and environmental conditions with the exception of an anti-correlation with mossy texture ($r_p = -.23$, $P < 0.05$) (Table 6 and Appendix F).

Carbonate (calcite) is not a quantitatively correlated with any of the texture types or an abundance amount suggesting pH, high amounts of dissolved CO₂, or some other factor effecting carbonate precipitation (Table 6 and Appendix F). Carbonate minerals are not representative of any conclusive conditions driving a subsurface basaltic ecosystem.

Zeolite minerals are strongly quantitatively correlated with overprinting ($r_p = .24$, $P < 0.05$) and a high abundance of biotic activity ($r_s = .36$, $P < 0.05$) (Figure 22, Table 6, and Appendix F) suggesting because zeolites are stable in saline fluids with a higher pH (Faure, 1998), perhaps higher salinities, and a higher pH could be an influence of biotic activity. Higher salinity could indicate prolonged water-rock interaction in older samples. Additionally, overprinting indicates multiple stages of alteration.

Oxidized minerals show a correlation with curvilinear ($r_s = .24$, $P < 0.05$) and long tunnels ($r_p = .28$, $P < 0.05$) (Figure 22, Table 6, and Appendix F) suggesting that oxygenated fluids could be a control of the biotic community creating these textures.

Microscopic sulfide minerals do not show a correlation (Table 6 and Appendix F) with biotic alteration textures or abundance and therefore was found to be an inconclusive indicator of environment.

5.2.6 Summary

Overall the environmental parameters did not show many quantitative correlations with biotic alteration. Some general trends are listed here. Abundance showed an increase with depth into basalt corroborating the conclusion that biotic alteration occurs through time. The correlation between curvilinear tunnels and sample age, temperature, and oxidized minerals remains somewhat enigmatic. Perhaps with age and a resurgence of hydrothermal activity curvilinear tunnels are made more prolific. Or perhaps they simply are created over time. Abundance is quantitatively correlated with sample depth and zeolite presence suggesting perhaps that a wide range of conditions could contribute to increased abundance. The similar correlation between overprinting, sample depth and zeolite presence could simply confirm the fact that overprinting is a superposition of alteration fronts and so inherently creates a high abundance of biotic alteration. The correlation between long tunnels and oxidized minerals could suggest that for long tunnels to occur, oxidizing conditions are necessary.

There are several explanations for the lack of clear directional correlations that point to one type of environment, young crust, reducing conditions etc. The oceanic crust is a complex environment with large gradients of fluids, heat, nutrients, and metals. This study only represents a small picture of the variables that could possibly influence the seafloor biosphere. In addition to the complexity of the seafloor environment there are ecological limitations. When using presence- absence data an assumption is made that the absence of a particular component of biotic alteration indicates the environment is uninhabitable (Elith and Leathwick, 2009). Other explanations could

include non-residency or predation and competition. More data is necessary to confirm that perhaps colonization simply has not taken place in certain locations.

5.3 Future work

1. The imperviousness of most of the biotic alteration textures and abundance to oxidizing conditions suggests they are capable of adapting to changing oxidation state. Collecting data on the oxidation state of the biotic alteration from many environments and then comparing the abundances of the individual textures could verify whether this is true. In particular, data collected directly adjacent to or within the individual textures instead of the hand sample shipboard observations used here would help to resolve this.
2. The response of a narrow range of biotic alteration textures to only a few environmental parameters suggests perhaps these are well adapted to changing conditions through time. This could be explained by the relationship between these modern textures and the Archaean analogs. If the modern ocean samples are derived from the same biotic mechanism that is creating the textures in the samples from the Archaean perhaps the microbial communities participating in texture formation evolved the ability over time to survive under variable conditions. In depth comparison between the modern examples and Archean analogs could reveal the conditions associated with the biotic textures in the early ocean crust. Correlating similar features in modern and Archean rocks, and determining the modern conditions more thoroughly could accomplish whether these are the same microbial systems.
3. This study did not reveal that many texture types were strongly correlated with conditions suggesting a wide range of adaptability or a diverse consortium of microbes participating in texture formation. To test which of these is true would require cultivating microbes on basaltic glass with known temperature, pH, and oxidation adaptations and investigating any physical evidence on the glass from the microbial community. Alternatively attempting to isolate a diverse range of

microbes from the ocean crust and evaluating the physical evidence produced on the glass could answer this.

4. Other possibilities for future microbial community and abundance studies include collecting more *in situ* data on the fluid chemistry, pH present with the samples for correlation with morphology as is common with marine sedimentary eudendolithic shell-boring bacteria (Cherci, et al., 2012).
5. Selecting samples along a vertical or horizontal transect could reveal environmental controls of biotic alteration. For example analyzing samples from specific locations, close to outcrops, or in areas of low sediment input, or collecting samples from fast and slow spreading ridges to investigate the contrast.
6. Long range studies are necessary as one of the primary limiting factors of culturing microbes from this environment for laboratory reproduction of textures is the low growth range due to limited nutrients and limited free energy (Konhauser, 2007; Perry et al., 2002).
7. Some secondary minerals were correlated with biotic alteration textures and high abundance, suggesting a relationship between biotic activity and water-rock interactions. The implications of this relationship could be meaningful to biogeochemical cycling and further investigation is needed to full understand the impact of biotic alteration on ocean chemistry. Secondary mineralization is a potential environmental indicator that can serve as a starting point. Further elucidating the relationship between secondary mineralization and biotic alteration could prove to be beneficial for selecting type locations to search for evidence of life on Mars.

The subsurface biosphere in the igneous crust is a complex system and the premise of that the textures have a biological origin has not been proven or disproven here. Because the sample size is 80, it is also difficult to extrapolate this to the whole crust. This study does however represent the first systematic global analysis of texture morphology and

abundance in relation to the seafloor conditions and is only one starting point of understanding the global phenomenon of the igneous subsurface biosphere.

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APPENDICES

Appendix A Continued. Biotic texture classification																												
Sample Number	1 granular	2 mossy	3 overprinting	4 empty	5 thin	6 convoluted	7 dark	8 curvilinear	9 long	knotted	kinked	short	network	buds, bubbles	smooth	tapered	wide	patterned	annulated	rough	crown	bumpy	ovoid bodies	close	mushroom	separate	simple	engorged
765D-5R-8	✓	✓	✓		✓		✓	✓																				
770B-16R-3-142	✓						✓	✓	✓	✓																		
770C-4R-1-14	✓						✓		✓												✓							
793B-105R-1-134			✓				✓																					
802A-58R-2-98	✓	✓		✓								✓		✓														
803D-69R-1-50	✓	✓	✓	✓	✓	✓			✓		✓							✓		✓								
803D-70R-2-117	✓	✓		✓	✓	✓		✓	✓	✓						✓								✓				
807C-92R-2-110	✓	✓	✓			✓	✓		✓							✓	✓			✓					✓			✓
833B-81R-2-73	✓	✓	✓	✓																								
836A-3H-7	✓	✓			✓	✓	✓	✓	✓	✓	✓			✓						✓	✓							
841B-49R-1-11	✓	✓		✓	✓			✓	✓					✓	✓	✓												
862C-5R-1-24	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓			✓	✓							✓					

Appendix A Continued. Biotic texture classification																													
Sample Number	1 granular	2 mossy	3 overprinting	4 empty	5 thin	6 convoluted	7 dark	8 curvilinear	9 long	knotted	kinked	short	network	buds, bubbles	smooth	tapered	wide	patterned	annulated	rough	crown	bumpy	ovoid bodies	close	mushroom	separate	simple	engorged	
1026B-3R1	✓	✓		✓	✓				✓			✓			✓		✓	✓											
1203A-19R-2	✓	✓	✓	✓											✓														
1203A-31R-1 118	✓	✓	✓		✓	✓	✓	✓	✓		✓				✓								✓						

Appendix B A list of the names describing textures in volcanic glass divided into those interpreting textures as resulting from biotic processes and those names interpreting the textures as resulting from abiotic processes.

<i>Names with biotic interpretation*</i>	<i>Reference</i>
Bioalteration	(McLoughlin et al, 2009; Staudigel et al., 2008)
Bioerosion	(McLoughlin et al., 2009)
Bio-generated tunnels	(Furnes et al., 2002)
Biogenic alteration	(Storrie-Lombardi & Fisk, 2004)
Biogenic corrosion	(Callot et al., 1987)
Biogenic etching	(Callot et al., 1987)
Bio-interaction with basalt glass	(Furnes et al., 2002)
Biomediated alteration	(Furnes et al., 2008)
Biologically mediated dissolution	(Thorseth et al., 1992)
Bioerosion	(McLoughlin et al., 2009)
Filamentous microfossils	(Furnes et al., 2004; Hofmann, 2001)
Ichnofossil	(Banerjee et al., 2007; McLoughlin et al., 2009; Walton 2005)
Microbial alteration	(Banerjee & Muelenbachs, 2003; Staudigel et al., 1995)
Microbial dissolution	(Thorseth et al., 1992)
Microbial etching	(Banerjee et al., 2006; McLoughlin et al., 2009)
Microbial ichnofossils	(Banerjee et al., 2007)
Microbially induced alteration	(Furnes et al., 2004)
Microbially influenced corrosion	(Drewello & Weissmann, 1997)
Microbial trace fossils	(French & Blake, 2012)
Tubular microcavities	(McLoughlin et al., 2010b)
Names without abiotic interpretation*	Reference
Alteration textures	(Banerjee & Muehlenbachs, 2003)
Channels	(Alt & Mata, 2000; Banerjee & Muehlenbachs, 2003)
Irregular altered zones	(Alt & Mata, 2000)
Irregular patches or tubes lining cracks	(Alt & Mata, 2000)
Micrometer dissolution channels	(Alt & Shanks, 2012)
Micro-textures	(McLoughlin et al., 2009)
Microtubules	(Furnes & Staudigel, 1999; Walton, 2008)
Pits, spots, and spherules	(Alt & Mata, 2000)
Tubular alteration	(Knowles et al., 2012; Staudigel et al., 2008)
Names are from studies of microtextures in basaltic glass from the ocean crust. The names are divided into those that imply a biotic origin and those that do not. An ichnotaxonomic classification scheme was created in a study conducted by McLoughlin, et al., 2010 and is recommended for a full a comprehensive list beyond biogenetic implications.	

<i>Appendix C. Six environmental parameters and abundance estimations for all samples</i>												
Sample Number	Ocean Basin	I Age (Ma)	II Sample depth into basalt (m)	III Overlying Sediment thickness (m)	IV Temperature (°C)	Abundance %	V. * Presence Sulfides	** Oxidized minerals	** Smectite	** Carbonate	** Zeolite	** Pyrite
105-41-3-46	Atlantic	156	4.2	623.3	28	80	✓			✓		
215-18-1-67	Pacific	60	23.5	155.5	11	100		✓	✓	✓		
240-7-1	Indian	53	2	190	13	60	✓		✓	✓		
266-23-1-64	Indian	21	0.3	370.3	36	100				✓	✓	
332B-20-2-106	Atlantic	3.5	300.5	148	36	70				✓		
335-5-3-7	Atlantic	16.5	3	454	50	70			✓	✓		
335-9-5-43	Atlantic	16.5	38.4	454	50	70			✓	✓		
337-15-2-77	Atlantic	31	17.8	113	11	60	✓	✓	✓	✓		
373A-3-3-128	Mediterranean Sea	4	11.3	270	69	100				✓	✓	
395A-17-1 83-85 #11	Atlantic	7	117.5	93	17	60	✓					

<i>Appendix C Continued. Six environmental parameters and abundance estimations for all samples</i>												
Sample Number	Ocean Basin	I Age (Ma)	II Sample depth into basalt (m)	III Overlying Sediment thickness (m)	IV Temperature (°C)	Abundance %	V. *Presence Sulfides	**Oxidized minerals	**Smectite	**Carbonate	**Zeolite	**Pyrite
395A-17-1-91	Atlantic	7	118.4	93	17	70						
395A-56-3 8-10 #1C	Atlantic	7	467.6	93	17	100					✓	
395A-58-2 112-115 #61	Atlantic	7	489.2	93	17	100	✓					
395A-58-2-117	Atlantic	7	489.2	93	17	90	✓					
395A-67-1-146	Atlantic	7	319.6	93	17	90	✓			✓	✓	
396-14-6 108-110 #17	Atlantic	15	1.7	125	16	60						
417D-66-6-35	Atlantic	120	342.9	343	17	100	✓		✓	✓		
418A-68-3 41-42 #3A	Atlantic	120	409	324	16	100	✓		✓	✓	✓	✓
418A-71-3-87	Atlantic	120	432.6	324	16	90	✓		✓	✓	✓	✓
418A-74-6 85-88 #2E	Atlantic	120	456	324	16	90	✓		✓	✓	✓	✓
418A-86-2-81	Atlantic	120	537.8	324	16	60	✓		✓	✓	✓	✓
442B-19-1-12	Pacific	18	144	292	31	50	✓				✓	
443-54-8-33	Pacific	15	26.8	475	54	70	✓			✓		

<i>Appendix C Continued. Six environmental parameters and abundance estimations for all samples</i>											
443-62-3-105	Pacific	15	89	475	54	90	✓			✓	
447A-18-2-34	Pacific	28.5	37.8	113	11	100	✓		✓	✓	✓
447A-21-1-124	Pacific	28.5	55.2	113	11	100		✓			✓
447A-31-3-118	Pacific	28.5	134.7	113	11	60	✓		✓		
447A-32-2-48	Pacific	28.5	141.5	113	11	80	✓		✓	✓	✓
448A-10-2-142	Pacific	25	2.9	290	27	80	✓				
448A-15-2-18	Pacific	25	239.2	290	27	90					
448A-39-2-32	Pacific	25	424.3	290	27	90	✓		✓		
449-16-1-23	Pacific	25	12.7	111	11	50	✓		✓		
469-50-2-17	Pacific	18	51.7	391	41	40	✓			✓	
472-14-1-26	Pacific	15	0.3	112	14	100					
472-15-1-102	Pacific	15	7.5	112	14	100				✓	
474A-48-1-92	Pacific	3.5	85	515	119	80	✓				
474A-48-1-92	Pacific	3.5	85	515	119	100	✓				
474A-48-1-92	Pacific	3.5	85	515	119	70	✓				
482D-11-2-32	Pacific	0.5	23.3	138		50			✓	✓	✓
483-21-2-34	Pacific	2.4	68.8	110	14	40			✓	✓	

<i>Appendix C Continued. Six environmental parameters and abundance estimations for all samples</i>											
504B-4-2	Pacific	5.9	7	274.5	23	60	✓		✓	✓	
520-31-1-18	Atlantic	14.3	1.7	449	28	30					
522B-5-1-105	Atlantic	35	7.9	154	10	100				✓	
543A-16-3-11	Atlantic	83	39.1	409	25	80	✓	✓		✓	
543A-16-7 60-64 #30	Atlantic	83	39.1	409	25	100	✓	✓			
556-4-4 108-112	Atlantic	28	9	471	27	60			✓	✓	
558-31-1	Atlantic	33	33.5	408	23	50	✓			✓	
559-2-2	Atlantic	15	9	249	17	70				✓	
562-9-1	Atlantic	15	64	268	18	40				✓	
597A-7-CC	Pacific	25	0.4	47.6	5	90		✓		✓	✓
655B-1-1-107	Mediterranean Sea	3.5	2.3	79.9	20	40				✓	
655B-12-1-34	Mediterranean Sea	3.5	107.2	79.9	20	60				✓	
713A-20R-2-89	Indian	48	69.3	107	8	50		✓		✓	
713A-20R-3	Indian	48	68.4	107	8	70	✓	✓		✓	
713A-20R-5-8	Indian	48	71	107	8	80	✓	✓		✓	
713A-20R-5-98	Indian	48	71	107	8	90	✓	✓		✓	
713A-20R-6	Indian	48	72.9	107	8	90	✓	✓		✓	
758A-71R-2	Indian	48	77.8	107	8	70	✓		✓	✓	

<i>Appendix C Continued. Six environmental parameters and abundance estimations for all samples</i>											
765C-63R-1-12	Indian	14 2	0.3	935. 6	69	70	✓	✓		✓	
765D-5-1-53	Indian	14 2	59.7	924	68	90	✓			✓	
765D-5-8-81	Indian	14 2	68	924	68	50	✓			✓	
765D-5R-1	Indian	14 2	59.2	924	68	70	✓			✓	
765D-5R-8	Indian	14 2	59.3	924	68	50	✓			✓	
765D-5R-8	Indian	14 2	59.3	924	68	90	✓			✓	
770B-16R-3-142	Pacific	42	4.1	420. 9	24	40	✓	✓		✓	
770C-4R-1-14	Pacific	42	28.8	423. 2	24	60	✓				
793B-105R-1-134	Pacific	32	181. 6	1403 .9	75	80			✓		✓
802A-58R-2-98	Pacific	11 2	7	509	34	90	✓			✓	
803D-69R-1-50	Pacific	65	0.42	630. 98	36	60	✓	✓			
803D-70R-2-117	Pacific	65	10.1 2	630. 98	36	40				✓	
807C-92R-2-110	Pacific	11 2	137. 1	1379 .7	89	10 0		✓	✓	✓	
833B-81R-2-73	Pacific	4.2	124. 9	952. 9	87	10 0	✓				✓
836A-3H-7	Pacific	1	4.3	22.8	6	30					
841B-49R-1-11	Pacific	40	16.9	212. 3	13	50					
862C-5R-1-24	Pacific	4.2	43.3	22.1	4	40		✓	✓	✓	
1026B-3R1	Pacific	3.5	35	250	26	20				✓	

<i>Appendix C Continued. Six environmental parameters and abundance estimations for all samples</i>											
1140A-26R-1 81-84	Indian	35	4	270	16	70				✓	
1140A-35R-1 31	Indian	35	67	270	16	70				✓	
1203A-19R-2	Pacific	73	16	462	27	50		✓		✓	
1203A-31R-1 118	Pacific	73	118	462	27	90		✓		✓	
<p>I. Age (Ma) values, II Basalt depth, III Overlying sediment thickness, and the **secondary mineralogy (V), with the exception of the sulfides were data obtained from the ODP and the DSDP. The first three environmental parameters were compiled in a previous study by Josef (2006) from the DSDP and the ODP volumes.</p> <p>*Sulfide observations (V) were made using thin sections of the samples.</p>											

Appendix D. Method of temperature calculation using heat flow modeling.

Cooling of the oceanic lithosphere can be modeled by conduction of heat away from the ocean lithosphere into the open ocean with some modifications (Stein and Stein, 1994). These modifications are necessary because several crustal processes inhibit, and promote, conductive cooling of the oceanic lithosphere. Heat flow is promoted when convective cooling by hydrothermal fluids occurs near the ridge crest (from 0 to 1 Ma) and when advective cooling occurs on the ridge flanks (1 Ma to 65 Ma). Heat flow is inhibited when mineral deposition reduces permeability and porosity within the crust (after about 65 Ma). These modifications have to be factored in when modeling heat flow in the ocean crust.

The theoretical heat flow is modified as follows. For equation (1) below: the lower limit of < 1 Ma accounts for hydrothermal circulation and advective heat transfer (Heberling et al., 2010; Stein and Stein, 1994). The upper limit of 55 Ma is a conservative estimate for modeling the 65 Ma sealing age to account for two factors. First this model is created from a global heat flow data set that when applied is only estimated to represent local heat flow, and second the effect of sediment hydrologically isolates the igneous crust at sediment thicknesses greater than about one to two hundred meters. The rate of this isolation effected by sediment thickness depends on age of the crust, tectonic evolution, basement structure, sediment source and deposition process (Divins, 2003).

Appendix D. Continued, temperature calculation from heat flow modeling
Temperature (T) was found using

Fourier's first law of heat conduction in 1 – D :

Q_{obs} (mW/m^2) is the heat flow per unit area

k is the thermal conductivity

$\frac{dT}{dz}$ is the change in temperature with depth

$$Q_{obs} = -k \frac{dT}{dz} \quad (5)$$

Assuming

$T_i 2^\circ C^*$, temperature at the **sediment**
/ **water** interface (Fisher and Harris, 2010)

Bottom water in the Mediterranean Sea is warmer than other ocean basins and
 T_i is assumed to be $10^\circ C$ (Goffredo and Dubinsky, 2013).

$$k_{sediment} = 1.2 \text{ W}/m^\circ C \text{ (Pribnow et al., 2000)}$$

Z is sediment depth and $z_i = 0$ at the sediment water interface

Solving for T_f , temperature at the **sediment** – **basement** interface,

$$\frac{dT}{dz} = \frac{Q_{obs}}{-k} = \frac{T_f - T_i}{-Z_f}$$

$$T_f = \frac{Q_{obs}}{k} + T_i \quad (6)$$

Appendix E Alteration mineralogy data mining method for ODP and DSDP volumes

Alteration minerals were counted as present when they occurred within one meter of the sample core location, or if mineralogy was recorded for the whole unit (Figure ***) this was recorded. Mention of alteration minerals was found primarily in the Shipboard Scientific party section in the summary of the drill hole where the core was divided up into lithologic units. The lithologic units were summarized and described in terms of individual sections in the unit. Figure 12 shows a page from a DSDP volume with relevant unit summary and mineralogy section highlighted.

Appendix E. Continued, Alteration mineralogy data mining method for ODP and DSDP volumes.

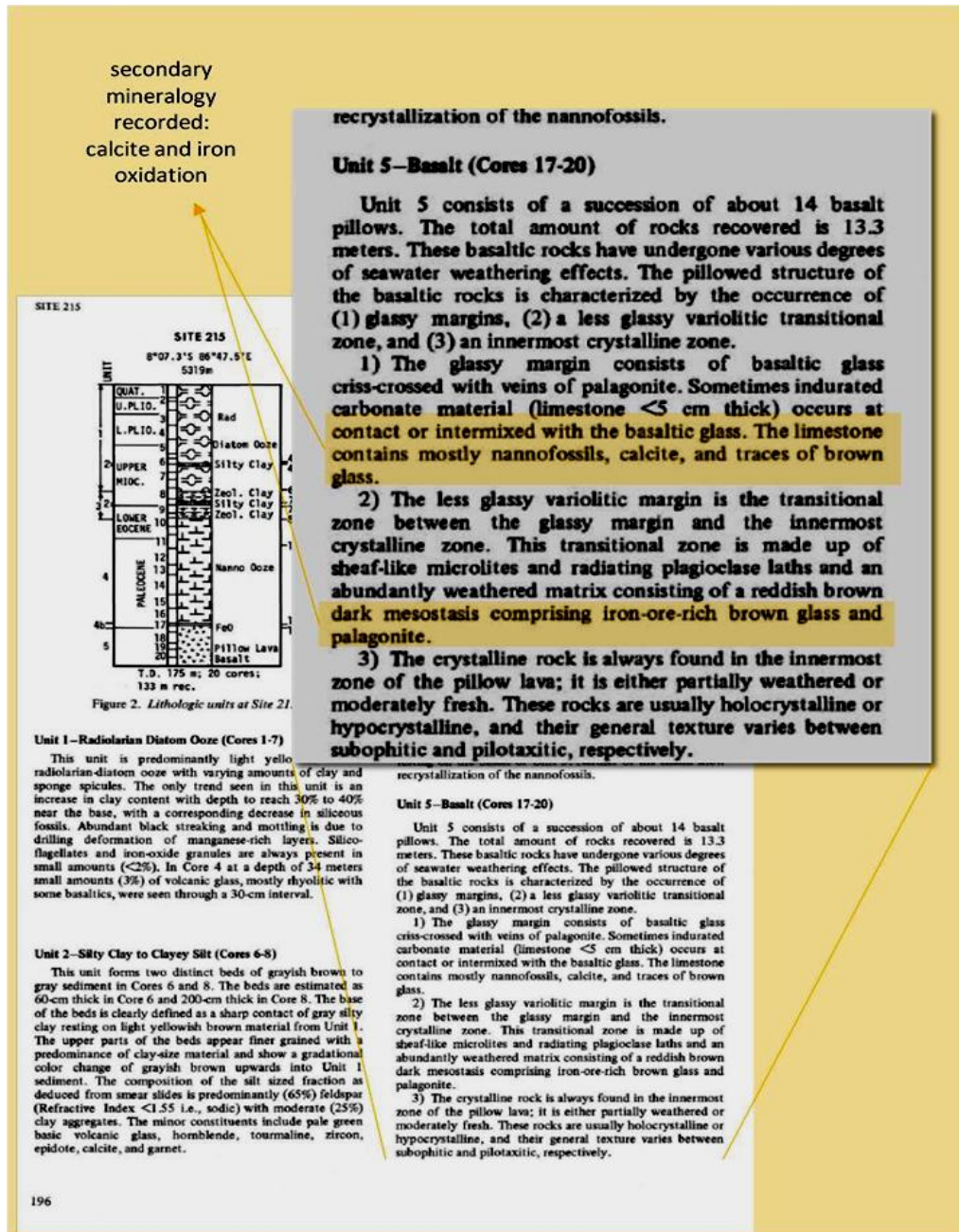


Figure 23. Sample page from volumes to show data mining method. Unit summaries were contained within the shipboard scientific party section. The highlighted portion was considered relevant information and the secondary mineralogy recorded was carbonate minerals and iron oxidation.

Appendix F. Statistical analysis of the correlations between the distinctive textures and the environmental parameters

The focus of these analyses was to determine whether correlations exist between biotic alteration of subseafloor volcanic glass and the environmental parameters associated with the rocks. Analyses were also conducted focusing on the association between abundance and distinctive textures. Correlation coefficients that measure the relationship between two variables were selected, Pearson product-moment correlation coefficient r_p , and Spearman rank correlation coefficient, r_s (Currell and Dowman, 2009; Zar, 1999). To determine whether there were statistically significant correlations between the parameters as outlined in the hypotheses Pearson's r_p or Spearman's r correlation coefficients were calculated using IBM SPSS Statistics v. 16.0 (Currell and Dowman, 2009; Zar, 1999). The correlation coefficients, as they are calculated in this study, represent the correlation strength of two variables, e.g., long tunnels and sample age, relative to the correlation strength of the other distinctive textures, e.g., mossy tunnels and sample age (Equation 1 and 2).

The values considered statistically significant for Spearman's ρ and Pearson's r were obtained by comparison to statistically significant critical values at the 95% confidence level ($P < 0.05$) and 99% confidence level ($P < 0.01$) for two-tailed hypothesis testing with $df = n - 2$, where $n = 80$ (Zar, 1999). These confidence levels were selected prior to the analysis. A value that falls in the 95% confidence level implies that the null hypothesis is false 5% of the time, or that the occurrence of correlation is random $< 5\%$ of the time (Zar, 1999). This means that the alternate hypotheses $H_{1(A-C)}$ are not proven true, rather they are not false.

The assumptions made were that the 80 samples are randomly selected; the distinctive texture and high abundance of biotic alteration occurrences are independent of one another. The degrees of freedom were 78, $n - 2$ where $n = 80$. In other words, a specific distinctive texture type occurrence is not dependent on the presence or absence of any of the other types. The relationships between the distinctive textures are not known and so

this is considered reasonable. Future analyses might take into account the relative influence of the distinctive texture types and a high abundance of biotic alteration. Similarly, the environmental parameters are assumed to be independent of each other. The exact relationship between the environmental parameter variables could be investigated with a multivariate approach in future studies. Pearson's measures a linear relationship and there may be other relationships between the biotic alteration and the environmental parameters.

The four continuous environmental parameters, sample age, sample depth into basalt, overlying sediment thickness and temperature, as well as biotic alteration abundance indicated strong positive skew with respect to a normal distribution. This is evident in the scatter plots of the continuous variables where majority of the samples have young sample age's and are from shallow depths (Figure 25). Therefore, Spearman's r_s was used in the correlations conducted for the four continuous environmental parameters

Pearson's correlation coefficient is calculated as follows:

$$r_p = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{(n-1)s_x s_y} \quad (1)$$

Spearman's rank correlation coefficient is calculated as follows:

$$r_s = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \quad (2)$$

Where the x values are the environmental parameters and the y values are the quantities of the samples that contain the respective distinctive textures. The average y value for the distinctive textures was calculated as the average number of times (49) the distinctive textures occurred out of all 80 samples. For reference, long tunnels, the least frequently occurring texture type, is found in 29 out of 80 samples and granular, the most frequently occurring texture type is found in 74 out of 80 samples.

Out of all analyses conducted, a total of nine significant correlations were indicated, with all analyses focusing upon the level of association between the biotic alteration textures and abundance in rocks and the environmental parameters.

Environmental parameters I-IV.

Sample age, depth into basalt, sediment thickness, and temperature

1. A positive, significant correlation was indicated between overprinting and sample depth into basalt; the presence of overprinting was associated with increased sample depth.
2. A significant, positive, correlation was also found between curvilinear samples and sample age; curvilinear samples were found to be significantly older.
3. A negative, and statistically significant correlation was found between curvilinear samples and temperature; curvilinear samples were significantly associated with lower temperatures.
4. A positive, and statistically significant correlation was indicated between biotic alteration abundance and sample depth into basalt; a greater sample depth was associated with significantly greater biotic alteration abundance.

Environmental parameter V. Secondary mineralogy

4. A significant positive correlation was found between biotic alteration abundance and the presence of zeolite; the presence of zeolite was associated with significantly greater biotic alteration abundance.
5. A statistically significant correlation was indicated between the presence of smectite and the sample being mossy. This correlation was found to be negative, with this result indicating that mossy samples were significantly less likely to contain smectite.
6. A significant and positive correlation was indicated between the presence of zeolite and overprinting. While weak, this correlation indicated that the presence of zeolite was associated with a significantly increased likelihood of overprinting.

7. The correlation between the presence of oxidized minerals and the sample being curvilinear was found to be positive, and statistically significant. This result indicates that curvilinear samples were significantly more likely to contain oxidized minerals.
8. A statistically significant, positive, and weak correlation was indicated between the presence of oxidized minerals and the sample being long. This result indicates that long samples were significantly more likely to contain oxidized minerals.
9. Overprinting and high abundance of a significant positive correlation.

Table 7 Significance correlations between Abundance and Distinctive Textures		
	<i>High abundance of biotic alteration</i>	
<i>Granular</i>	0.1	
<i>Mossy</i>	-0.12	
<i>Overprinting</i>	0.24*	Correlation is positive and weak, overprinting is significantly associated with increased abundance
<i>Empty</i>	0.08	
<i>Thin</i>	0.02	
<i>Convoluted</i>	-0.05	
<i>Dark</i>	-0.08	
<i>Curvilinear</i>	0.00	
<i>Long</i>	-0.01	
<p><i>All analyses conducted consisted of Spearman's correlations r_s</i></p> <p><i>Note. *$p < .05$, **$p < .01$</i></p> <p><i>df = n-2, where n=80</i></p>		

Table 8 Significance correlations between distinctive textures, biotic abundance and environmental parameters.										
	<i>Abundance+</i>	<i>Granular</i>	<i>Mossy</i>	<i>Overprinting</i>	<i>Empty</i>	<i>Thin</i>	<i>Convolved</i>	<i>Dark</i>	<i>Curvilinear</i>	<i>Long</i>
<i>(I)Sample Age+</i>	0.16	0.03	0.06	0.11	0.07	0.19	-0.03	-0.09	0.25*	0.14
<i>(II)Sample depth into basalt+</i>	0.27*	-0.01	-0.02	0.29**	0.05	0.10	0.12	-0.05	-0.04	0.01
<i>(III) Overlying sediment thickness+</i>	0.05	0.01	.07	0.01	0.05	-0.06	-0.09	-0.05	-0.1	-0.07
<i>(IV) Temp. +</i>	0.05	-0.06	0.06	0.00	-0.04	-0.17	-0.14	0.04	-0.23*	-0.12
<i>(V) Secondary Minerals</i>										
<i>Smectite</i>	0.02	-0.00	-0.23*	0.05	-0.06	-0.1	0.04	0.07	-0.12	-0.13
<i>Carbonate (Calcite)</i>	-0.06	0.07	-0.07	-0.04	0.04	0.06	0.03	-0.01	0.18	0.02
<i>Zeolite</i>	0.36*	-0.08	-0.11	0.24*	0.1	-0.07	-0.11	-0.08	-0.2	-0.03
<i>Oxidized Minerals</i>	0.08	0.06	0.09	0.09	0.11	0.15	0.18	-0.09	0.24*	0.28*
<i>Pyrite</i>	0.07	-0.10	0.14	0.05	0.12	-0.04	-0.02	-0.11	-0.2	-0.19
<i>Sulfides</i>	0.16	0.17	-0.09	0.18	0.17	0.14	0.10	-0.14	0.1	0.1
<p>Note: *$P < .05$, **$P < .01$; $df = n-2$, where $n=80$</p> <p>⁺Spearman's correlations.</p> <p>Numbers are correlation coefficients, either Pearson's r_p or Spearman's r_s where indicated. These coefficients can assume negative and positive values that vary between 0 and 1, where 1 indicates a perfect correlation and 0 indicates no correlation.</p>										

