



**INTEGRATED USE OF DRY AZOLLA BIOMASS AND  
INORGANIC NITROGEN FERTILIZER FOR SOIL AND TOMATO  
(*Solanum Lycopersicon* L.) PRODUCTIVITY IN SELECTED DISTRICTS  
OF SIDAMA REGION, ETHIOPIA**

**Ph.D. DISSERTATION**

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**HAWASSA UNIVERSITY  
COLLEGE OF AGRICULTURE**

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**HAWASSA, ETHIOPIA**

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(*Solanum Lycopersicon* L.) PRODUCTIVITY IN SELECTED DISTRICTS  
OF SIDAMA REGION, ETHIOPIA**

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**A Ph.D. Dissertation Submitted to the School of Plant and Horticultural  
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**College of Agriculture**

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**SCHOOL OF GRADUATE STUDIES HAWASSA UNIVERSITY**

**ADVISOR'S APPROVAL SHEETS**

This is to certify that the thesis entitled “**Integrated Use of Dry Azolla Biomass and Inorganic Nitrogen Fertilizer for Soil and Tomato(*Solanum lycopersicon* L.) Productivity in Selected Districts of Sidama Region , Ethiopia ”** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.) with specialization in **Soil Science**, the graduate program of the **School of Plant and Horticultural Sciences**, and has been carried out by **Habtamu Alemayehu Lorebo** (ID.No PhD SoScR/0005/13), under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby can submit the dissertation to the school.

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We, the undersigned, members of the Board of Examiners of the final open defense by **Habtamu Alemayehu Lorebo** have read and evaluated his dissertation entitled “**Integrated Use of Dry Azolla Biomass and Inorganic Nitrogen Fertilizer for Soil and Tomato(*Solanum lycopersicon* L.) Productivity in Selected Districts of Sidama Region, Ethiopia**” and examined the candidate. This is, therefore, to certify that dissertation has been accepted in partial fulfillment of the requirements for the Doctor of Philosophy degree (PhD) in Soil Science.

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Final approval and acceptance of the dissertation is contingent upon the submission of the final copy of the dissertation to the School of Graduate Studies (SGS) through the Department/School Graduate Committee (DGC/SGS) of the candidate’s department.

Stamp of SGS Date: \_\_\_\_\_

## STATEMENT OF THE AUTHOR

I, the undersigned, hereby declare that this PhD dissertation is my original work, has not been presented for a degree at any other university, and that all sources of materials used for the study have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for a PhD degree at Hawassa University and is deposited at the University library to be made available for users under the library's rules. I declare that this dissertation has not been submitted to any other institution for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission, provided accurate acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the School of Graduate Studies when, in his judgment, the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author and advisors of this dissertation.

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## **BIOGRAPHICAL SKETCH**

The author, Habtamu Alemayehu, was born on 20 November 1992 in Mudula town of Central Ethiopia Region. He attended his primary and junior education at Gidansonga School and his secondary and preparatory education at Mudula Secondary and Preparatory School from 2005 to 2008. In 2009, he joined University and graduated with a BSc degree in Rural Development in 2011. He began his professional career at Tembaro Special District, Agriculture and Natural Resource Development Office, where he worked as a soil and water conservation. Then, he joined Hawassa University MSc studies in Soil Science in 2015 and graduated with a MSc degree in Soil Science in 2018. On March 22, 2018, he then joined Samara University as a lecturer and researcher until he returned to the Hawassa University, School of Plant and Horticultural Science of Hawassa to pursue his PhD studies in Soil science in 2020.

## **ACKNOWLEDGMENTS**

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## **DEDICATION**

This PhD dissertation work is dedicated to: my beloved wife Sintayehu Mulu, father and mother Alemayehu Lorebo and Amarech Meskele and my son Diyara Habtamu

**APPROVAL SHEET**  
**HAWASSA UNIVERSITY**  
**SCHOOL OF GRADUATE STUDIES**

This is to certify that the dissertation research entitled “**Integrated Use of Dry Azolla Biomass and Inorganic Nitrogen Fertilizer for Soil and Tomato (*Solanum lycopersicon* l.) Productivity in Selected Districts of Sidama Region, Ethiopia**” open defense by **Habtamu Alemayehu**. The dissertation accepted in the partial fulfillment of the requirement for the degree of Doctor of Philosophy of Science in **Agriculture** with specialization in **Soil science** in the graduate program of the **School of Plant and Horticultural Sciences**.

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## LIST ACRONMYS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AE	Agronomic Efficiency
Ava.P	Available Phosphorus
ANOVA	Analysis of Variance
ARE	Apparent Recovery Efficiency
AS	Apparent Specific Gravity
ATA	Agricultural Transformation Agency
AWC	Available Water Content
BD	Bulk Density
BNF	Biological Nitrogen Fixation
BoFB	Finance and Economic Development Bureau
C/N	Carbon to nitrogen ratio
CEC	Cation Exchange Capacity
CRD	Complete Randomized Design
CSA	Central Statistical Agency
DA	Dry Azolla
DAT	Days after Transplanting
DTPA	Diethylene Triamine Penta Acetic Acid
EC	Electrical Conductivity
EMA	Ethiopian Mapping Authority

ENMA	Ethiopian National Meteorological Agency
ETB	Ethiopian Birr
EDTA	Ethylene Diamine Tetra acetic Acid
FAO	Food and Agriculture Organization
FC	Field Capacity
FNU	Fruit Nitrogen Uptake
FYM	Farm Yard Manure
GLM	General Linear Model
ha	Hectare
NER	Nitrogen efficiency Ratio
ICAP	Inductively Coupled Plasma Atomic emission Spectrometry
IFPRI	International Food Policy Research Institute
INM	Integrated Nutrient Management
IRRI	International Rice Research Institute
ISFM	Integrated Soil Fertility Management
IUSS	International Union of Soil Sciences
LSD	Least Significant Difference
m.a.s.l	meters above sea level
m	meter
MARC	Melkassa Agricultural Research Center
MoAR	Ministry of Agriculture and Rural Development

NER	Nitrogen Efficiency Ratio
NUE	Nitrogen Use Efficiency
OC	Organic Carbon
PD	Particle Density
PGPR	Plant Growth Promoting Rhizobacteria
PUE	Physiological Use Efficiency
PWP	Permanent Wilting Point
RCBD	Randomized Complete Block Design
RGR	Relative Growth Rate
RSG	Reference Soil Group
PBS	Percentage Base Saturation
SAS	Statistical Analysis Software
SD	Standard Deviation
SGS	School of Graduate Studies
SNU	Shoot Nitrogen Uptake
SNNPRS	Southern Nation Nationality People Regional State
SOM	Soil Organic Matter
SON	Soluble organic nitrogen
SSDS	Soil Survey Division Staff
SSS	Soil Survey Staff
T	Tone

TN	Total Nitrogen
TP	Total Porosity
TSP	Triple Superphosphate
UE	Utilization Efficiency
USDA	United States Department of Agriculture
WRB	World Reference Base

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### **LIST OF ARTICLES AND MANUSCRIPTS**

This PhD dissertation is composed of the following manuscripts, from which two are published, one is submitted and under review and the remaining manuscript is ready for submission to reputable journal.

**Paper I. Published article**

Alemayehu, H., Haile, W., and Kiflu, A. (2025). Characterization and classification of soils at Jara Gelalicha and Aruma areas, Sidama region, southern Ethiopia. *Frontiers in Environmental Science sec. soil Processes*, <https://doi.org/10.3389/fenvs.2025.1569469>.

**Paper II. Manuscript submitted and under review**

Habtamu Alemayehu, Wassie Haile, Alemayehu Kiflu and Ashenafi Haile. Influence of Azolla dry biomass and inorganic nitrogen Fertilizers on soil chemical properties, yield and yield components of tomato under greenhouse conditions. Plos one (under review)

**Paper III. Manuscript ready for submission**

Habtamu Alemayehu, Wassie Haile, Alemayehu Kiflu and Ashenafi Haile. Integrated application of Azolla dry biomass and nitrogen fertilizer enhancing soil fertility, nitrogen use efficiency, and tomato productivity under irrigation conditions in Hawassa Zuria and Wondo Genet districts, Sidama region.

**Paper IV. Published article**

Alemayehu, H., Haile, W., Kiflu, A., and Haile, A. (2025). Effects of Azolla dry biomass on nitrogen mineralization in soils of Hawassa Zuria and Wondo Genet districts, southern Ethiopia, incubated under laboratory conditions. *International Journal of Agronomy*, 2025(1), 9315906. <https://doi.org/10.1155/ioa/9315906>.

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**General Abstract**

*Declining soil fertility and insufficient fertilizer application can limit crop yield. Integrating Azolla used as a source of organic nitrogen, with inorganic nitrogen fertilizers presents a promising strategy to improve soil health and crop productivity in tropical agroecosystems. This study was initiated to characterize soil, evaluate the effects of integrated application of Azolla dry biomass and inorganic nitrogen fertilizer on soil nitrogen mineralization, yield and nitrogen use efficiency of tomato in Hawassa Zuria and Wondo Genet, Sidama Region. The research conducted included soil characterization, through greenhouse, field and laboratory experiments with four specific objectives: (i) to characterize and classify soils at experimental sites, (ii) to determine the influence of Azolla dry biomass and inorganic N-fertilizer applications on selected soil chemical properties and as well as yield and yield components of tomatoes under greenhouse conditions, (iii) to evaluate the integrated effects of Azolla dry biomass and inorganic nitrogen fertilizer on soil chemical properties, nitrogen use efficiency, growth and yield of tomatoes, and (iv) determine the effects of Azolla dry biomass on soil nitrogen mineralization under controlled conditions. The first experiment, aimed to characterize and classify soils at experimental sites. A representative pedon was opened at each site to a depth of 2 x 2 x 2 m, and the profiles were described using the Guidelines for Field Soil Descriptions (FAO, 2006). A total of 12 disturbed and 12 undisturbed soil samples were collected from each diagnostic horizon at Jara Gelalicha, Hawassa Zuria and at Aruma, Wondo Genet locations. The soil analysis results showed that the surface horizon textural class of the pedon of Jara Gelalicha is sandy loam, whereas the textural class of the pedon at Aruma is sandy clay loam. Based on the World Reference Base for Soil Resources (WRB), the soils of the study areas were classified as Eutric Cambisols (Loamic) in the Jara Gelalicha area and Eutric Andosols (Loamic) in the Aruma area. Generally, the site-specific soil characterization and classifications could offer crucial information for designing soil management options to increase soil productivity. The second experiment, aimed to determine the influence of Azolla dry biomass and inorganic nitrogen fertilizer on soil chemical properties, yield and yield components of tomato under greenhouse. The treatments included four levels of Azolla dry biomass (control (0), 25, 50, and 75 g pot<sup>-1</sup>) and four levels of inorganic N*

fertilizers (0 (non-fertilized), 0.23, 0.46, and 0.69 g pot<sup>-1</sup>). The pot experiments were set up using factorial combinations within a completely randomized design (CRD) with three replications. In a greenhouse pot experiment, the interaction between Azolla dry biomass and inorganic nitrogen had a significant impact on various plant characteristics. The best results were achieved with the combined application of 75 g Azolla pot<sup>-1</sup> and 0.69 g pot<sup>-1</sup> of N, resulting in the highest marketable fruit yields at the Hawassa Zuria and Wondo Genet locations, which were 1088.43 g/plant and 833.73 g/plant, respectively. Field research is required to confirm the greenhouse findings and provide appropriate recommendations. Therefore, a third experiment, aiming to evaluate the integrated use of Azolla dry biomass and inorganic N fertilizers on soil chemical properties, growth and yield components, and nitrogen uptake and nitrogen use efficiency of tomato production was conducted under field conditions. Two-year field experiments were conducted in the Hawassa Zuria and Wondo Genet districts of the Sidama region. The experiment took place over two years in (2022/23 and 2023/24), a factorial combination of four rates of dry Azolla biomass (0, 5, 10, and 15 t ha<sup>-1</sup>) as an organic nitrogen source and four rates of inorganic N fertilizer (0, 46, 92, and 138 kg N ha<sup>-1</sup>) was evaluated. The treatments were arranged in a randomized complete block design (RCBD) with three replications. The combined two-year data showed that the highest marketable fruit yields in the Hawassa and Wondo Genet locations were 23.76 t ha<sup>-1</sup> and 26.35 t ha<sup>-1</sup>, respectively. This was achieved by adding 15 t ha<sup>-1</sup> of Azolla biomass and 138 kg ha<sup>-1</sup> of nitrogen. It is integrated application of 15 t ha<sup>-1</sup> of Azolla and 138 kg N ha<sup>-1</sup> is recommended for tomato production in both districts. The combined application of 15 t ha<sup>-1</sup> and 138 kg ha<sup>-1</sup> led to the highest fruit N uptake (1093.48 kg ha<sup>-1</sup> in Hawassa Zuria and 1486.94 kg ha<sup>-1</sup> in Wondo Genet, while the control treatment had the lowest. The final experiment was aimed to determine the effects of Azolla dry biomass rates on soil nitrogen mineralization under controlled conditions. In addition, an incubation experiment using a completely randomized design (CRD) with three replications was conducted to determine the combined application of four rates of Azolla dry biomass (0, 15, 30, and 45 g pot<sup>-1</sup>) and eight incubation periods (0, 7, 14, 21, 28, 42, 49, and 56 days of incubation). The results showed that the highest nitrogen mineralization occurred after 42 days of incubation with 45 g kg<sup>-1</sup> of Azolla in soils from both locations. The highest total mineralized N was found after 42 days of incubation in Hawassa Zuria and Wondo Genet (39.88 and 48.57 mg kg<sup>-1</sup> soil, respectively). Field research is essential to confirm the laboratory findings and provide appropriate recommendations.

**Keywords:** Andosols, chemical properties, period of incubation, soil horizons, tomato productivity.

## CHAPTER ONE

### 1. GENERAL INTRODUCTION AND DESCRIPTION OF THE STUDY AREAS

#### 1.1. Background and Justification

Ethiopia long-term food security and economic growth mostly depend on agriculture (Neglo et al., 2021). Additionally, small-scale farming makes up the majority of Ethiopia's agricultural sector, which is the main driver of the country's economy. Low soil fertility and crop nutrient imbalances are major challenges preventing Ethiopian farmers from achieving high agricultural productivity (Yigezu, 2021). Declining soil fertility is a serious limitation to crop production in Ethiopia (Zelleke et al., 2019). The primary causes are loss of organic matter, macro and micronutrient depletion, soil salinity, soil acidity, topsoil erosion, and deterioration of physical soil properties (Saniga et al., 2023). Different studies conducted in Ethiopia in the past few years have demonstrated the deficiency of nitrogen (N) (Erkossa et al., 2022). While some practices that could address these issues are occasionally used independently, a comprehensive package of interventions needs to be popularized throughout the country to effectively improve soil health and potentially increase crop yields (Shah and Wu, 2019).

Soil characterization is essential for understanding the physical, chemical, mineralogical, and biological properties of soil, as well as for organizing knowledge and facilitating the transfer of experience and technology (Chekol and Mnalku, 2012; Adhanom and Teshome, 2016). It is fundamental for classifying, and gaining the best understanding of the environment (Onyekanne et al., 2012; Yitbarek et al., 2018). According to Dinssa and Elias (2021), soil classification is a vital tool for classifying soil, identifying soil types, managing property, and transferring research findings. Soil categorization is critical for understanding soil behavior and establishes the foundation for effectively assessing soil suitability and maintaining fertility (Desalegn et al., 2014). To achieve the highest level of crop productivity, a comprehensive understanding of a nation's soil nature and characteristics is crucial for managing it according to its potential (Regassa et al., 2023).

Nitrogen is the most limiting factor in crop production, essential for growth, protein manufacture, and yield (Liliane and Charles, 2020). Abebe et al. (2022) reported that low fertilizer utilization and soil nutrient depletion are major reasons for low agricultural productivity in African countries, particularly in Ethiopia. Among these factors, the application of the optimum nitrogen

fertilizer rate is a main determinant that significantly affects the growth and yield of tomatoes in Ethiopia (Tesfaye et al., 2024). Tomatoes are highly responsive to nitrogen fertilizer application, especially when nitrogen availability is limited, and the timing of application is critical (Beyene and Mulu, 2019). Li et al. (2023) reported that nitrogen promotes vegetative growth and fruit yield, favors fruit development (when applied at a later growing stage), and has a dramatic effect on tomato growth and development. Continuous use of inorganic sources of nitrogen is a major source of contamination and pollution in aquatic environments (Prakash and Khanam, 2021). Additionally, the costs of these fertilizers are high. Therefore, organic fertilizers such as Azolla are good alternatives to inorganic fertilizers. More than 85% of Ethiopian soils contain insufficient nitrogen for sustainable and profitable crop production (ATA, 2012). Azolla fern is prevalent worldwide and has been used for centuries in paddies as a nitrogen source. However, its use declined with the manufacturing of inorganic fertilizers after the industrial revolution (Dixon, 2018).

Nitrogen mineralization is the process by which organic N is converted to plant-available inorganic forms (Drame, 2024). The rate of supply of available N generated by N mineralization involves the microbial conversion of more complex organic N into simpler available mineral-N forms ( $\text{NH}_4^+$ -N +  $\text{NO}_3^-$ -N). This implies that soil microorganisms exert significant influences on ecosystem functions by regulating litter decomposition and nutrient cycling (Abera et al., 2012). The rates of soil N mineralization and nitrification not only govern the availability of mineral N for plant growth but also indicate the ability of soil to return N (Li et al., 2019). In general, studying Azolla as an organic fertilizer helps determine how quickly it decomposes and releases nitrogen to meet crop needs.

Tomato (*Solanum Lycopersicon* L.) is one of the most important and widely grown vegetables in the world (Kumar et al., 2020). It is a member of the family Solanaceae, and it is a standout amongst the most broadly eaten vegetables in the world, which famously comes from the way that they can be eaten alone or as an ingredient in numerous recipes (Hyman, 2019). It is more important in a variety of dishes as a raw, cooked, or processed product than any other vegetable (Temgire et al., 2021). Tomato production in Ethiopia is approximately 26.5% of the vegetable production area. Ethiopia's average tomato yield is only  $8 \text{ t ha}^{-1}$ , far below the world average of  $34 \text{ t ha}^{-1}$  (FAOSTAT, 2012), and cultivated area has declined from 6,298.63 ha in 2016/17 to 5,235.19 ha in 2017/18 (Beyene and Mulu, 2019). However, the total production and productivity

in Ethiopia is far below the average of major producers in Africa (Yeshiwas et al., 2016). However, the total production and productivity in Ethiopia is far below the average of major producers in Africa (Yeshiwas et al., 2016). It is largely cultivated under irrigated conditions in the Rift Valley area of Ethiopia (Teklu, 2017). It is a profitable cash crop for smallholder farmers in Ethiopia. Nevertheless, the national average tomato fruit yield is often low ( $19.8 \text{ t ha}^{-1}$ ) compared even to the neighboring country, Kenya ( $23.2 \text{ t ha}^{-1}$ ) (Biramo et al., 2019). However, tomato production is highly constrained by several factors, especially in developing nations like Ethiopia. In Ethiopia, farmers get lower yields mainly due to inadequate fertilizers applications, diseases and pests (Etissa et al., 2013). Hasnain et al. (2020) reported that fruit yield in tomatoes is highly influenced by the rates of N fertilizers applied.

Azolla is a rapidly growing aquatic pteridophyte that forms a symbiotic relationship with the cyanobacterium *Anabaena azollae*, enabling the fixation of atmospheric nitrogen (Akhtar et al., 2021). When incorporated into the soil before or after crop transplanting, Azolla enhances soil fertility and reduces the reliance on inorganic fertilizers. Beyond nitrogen enrichment, its application increases soil microbial diversity, supporting long-term soil health (Akhtar et al., 2021). Azolla also contributes to environmental sustainability by sequestering atmospheric carbon (Bharali et al., 2021). Notably, it is among the fastest-growing plants, capable of doubling its biomass within 5–10 days (Brouwer et al., 2015). During decomposition, Azolla produces humic substances that positively influence soil fertility (Adnyana et al., 2025). The organic matter derived from decomposed biomass promotes microbial development, significantly increasing soil organic nitrogen (Yadav et al., 2014). In paddy systems, this fern releases biologically fixed nitrogen to crops through its cyanobacterial symbiont (Bocchi et al., 2010). The Azolla–*Anabaena* complex can fix approximately  $1.1 \text{ kg N ha}^{-1} \text{ day}^{-1}$ , supplying  $30\text{--}60 \text{ kg N ha}^{-1}$  to vegetable crops within 20–25 days (Adhikari et al., 2020). Azolla increases N, P, K, and micronutrient contents in the soil, enhancing microbial activity, porosity, soil water-holding capacity, and water infiltration rate (Mishra et al., 2013; Jumadi et al., 2014). The most common mode of Azolla application in the field is as manure or as a dual crop along with rice. Azolla decomposes within 8–10 days, releasing fixed nitrogen (Yadav et al., 2014). Azolla also improves N fertilizer efficiency (Macale and Vlek, 2004). Studies have shown that Azolla use increases yield equivalent to applying  $60\text{--}80 \text{ kg N ha}^{-1}$ , accelerates nitrogen mineralization in the soil, improves soil physical and chemical conditions, and increases soil organic matter (Subedi et al.,

2015), thus enhancing productivity and crop performance (Alim, 2012). Azolla incorporation increases crop nitrogen recovery by 49-64% and decreases N loss by 26-48% (Yao et al., 2018a). Several studies have shown the potential effect of Azolla application to improve soil chemical and physical properties (Maswada, et al., 2021; Seleiman et al., 2022). Al-Hamdawiet et al. (2020) noted that incorporating Azolla into the soil improves soil structure due to its high productivity, providing large quantities of organic matter. Organic fertilizers like Azolla not only increase crop productivity but also enhance long-term soil fertility. But in Ethiopia, there are few studies in the literature which demonstrate the effects of integrated application of Azolla dry biomass and nitrogen fertilizers on soil chemical properties and tomato yield (Zelege et al., 2024). Compared to inorganic nitrogen fertilizer, Azolla is an organic nitrogen source used as a fertilizer in various countries around the world. The incorporation of this organic nitrogen source alongside inorganic nitrogen is crucial for increasing tomato yield. Therefore, Azolla serves as an alternative organic fertilizer for significant nitrogen supplementation, particularly in developing countries. There have been numerous reports on the effects of Azolla leaf extract as a nitrogen source for rice (Yadav et al., 2014; Agbagba et al., 2018), other cereals (Simanungkalit, 2004), Chinese kale (Barus et al., 2018), tomatoes (Hanafy and El-Emery, 2018), and other vegetable crops (Dwi, 2017). Despite the potential benefits of using Azolla for sustainable crop production and improving soil physico-chemical properties, its potential as an organic nitrogen source for tomato cultivation in Ethiopia has not been fully explored.

Farmers in these regions frequently encounter challenges such as soil fertility decline, reduced crop yields, environmental degradation, and diminishing returns on agricultural inputs. These issues are largely associated with inadequate nitrogen application, often below recommended levels, alongside the high cost and inconsistent availability of inorganic fertilizers, which can delay their use in crop production. In contrast, organic fertilizers are generally more accessible and affordable for farmers (Kashem et al., 2015). Current research emphasizes the importance of integrated soil amendment strategies, as single-source inputs cannot sufficiently resolve underlying soil problems. Although inorganic fertilizers may temporarily enhance tomato productivity, they fail to sustain long-term soil fertility due to nutrient depletion through repeated crop harvesting (Sigaye, 2025). Integrated applications of Azolla dry biomass and inorganic nitrogen fertilizer have shown promise, but there is limited research on their combined effects on soil properties, nitrogen uptake and use efficiency, tomato yield and yield components in Hawassa Zuria and Wondo Genet districts, Sidama region. Developing locally adaptable

strategies that integrate these soil amendments is essential for restoring soil productivity and improving tomato yields in the region. In response to these challenges, this study was carried out to fill the knowledge gaps and support evidence-based in the Hawassa Zuria and Wondo Genet districts of southern Ethiopia. Therefore, this experiment was designed first to characterize and classify the soils of experimental sites. Second, the experiment aimed to determine the influence of Azolla dry biomass and inorganic N fertilizer on soil chemical properties and yield of tomato under greenhouse conditions. Third, the experiment focused to evaluate the integrated application of Azolla dry biomass and inorganic N fertilizers enhancing soil fertility, tomato productivity and nitrogen use efficiency under irrigation conditions. Finally, to determine the effects of different rates of Azolla dry biomass on soil nitrogen mineralization incubated under control conditions.

## **1.2. Objectives and hypotheses of the study**

The general objective of the study was to characterize the soils, effects of integrated application of Azolla dry biomass and inorganic nitrogen fertilizer for soil and tomato productivity in Hawassa Zuria and Wondo Genet districts, Sidama Region

The specific objectives of the study were:

1. To characterize and classify the soils of experimental sites
2. To determine the integrated effects of Azolla dry biomass and inorganic N fertilizers on soil properties and growth and yield of tomato under greenhouse conditions
3. To evaluate the integrated effects of Azolla dry biomass and inorganic N-fertilizers enhancing soil fertility ,tomato productivity and nitrogen use efficiency under irrigation conditions
4. To determine the effects of Azolla dry biomass rates on soil N mineralization in soils collected from Hawassa Zuria and Wondo Genet districts incubated under laboratory conditions

In light of the specific objectives articulated above, the study hypotheses are:

1. The soils of the experimental sites differ significantly in their physical, chemical, and morphological properties, leading to distinct soil classifications
2. Integrated use of Azolla dry biomass and inorganic N fertilizers can improve soil chemical properties and enhance tomato yield under greenhouse conditions

3. An integrated application of Azolla dry biomass and inorganic N fertilizers has effects on soil fertility, crop productivity and nitrogen use efficiency under irrigation field conditions.
4. The Azolla dry biomass rates and incubation periods significantly affect the soil nitrogen mineralization under laboratory conditions.

### **1.3. General Materials and Methods**

#### **1.3.1. Description of the Study Areas**

The study was conducted in farmers' fields in Jara Galalicha Kebele in Hawassa Zuria district and Aruma Kebele in Wondo Genet district of the Sidama Regional State, Southern Ethiopia. Jara Galalicha Kebele is located 290 km south of Addis Ababa and 16 km from Hawassa town. Geographically, the area lies at 7°0'13.7" to 7°48'11.5" N and 38°21'21.1" to 38°63'30.5" E, with a mean altitude of 1712 meters above sea level (masl) and slope ranges from 0.2 to 0.5 % (gentle). It receives a mean annual rainfall of 952 mm with mean minimum and maximum temperatures of 12.1°C and 26.7°C, respectively (Hawassa Meteorological Center, 2018). In the Hawassa Zuria, the soil texture is sandy loam, and the soil type is Cambisols. The major crops grown in the study area are maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* L.), wheat (*Triticum aestivum* L.), enset (*Ensete ventricosum* (Welw.)), beetroot (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), sweet potato (*Ipomoea batata* L.), coffee (*Coffea arabica*), khat (*Catha edulis* L.), and chili (*Capsicum annum* L.).

Aruma Kebele, in the Wondo Genet district, is located 264 km south of Addis Ababa and 27 km southwest of Hawassa town. Geographically, the area is located at 6°16' to 7°45' N and 38°31' to 38°63' E. The altitude of the district ranges from 1001 to 1780 meters above sea level. The mean annual rainfall in the district ranges from 801 to 1221 mm, and the mean annual temperature ranges from 17.6 to 22.5°C (SNNPRS, BoFED, 2018). In the Wondo Genet, the soil texture is sandy clay loam, and the soil type is Andosols. The major crops grown in the study area are khat (*Catha edulis* L.), sugarcane (*Saccharum officinarum* L.), maize (*Zea mays* L.), enset (*Ensete ventricosum* (Welw.) Cheesman), coffee (*Coffea arabica*), cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* L.), and potato (*Solanum tuberosum* L.). Both locations exhibit a landscape characterized by a mix of agricultural land, natural vegetation, and forest cover. The

soil pedons of the experimental sites were excavated in the cultivated land. The map of the study areas is presented in Figure 2.1. The ten-year annual climate data (2013-2022) for the Hawassa Zuria district and the Wondo Genet district are presented in Figures 2.2 and 2.3, respectively.

The field experiments were conducted for two consecutive years in the winter seasons from 2022 to 2024 at two selected locations in the Hawassa Zuria and Wondo Genet districts of Sidama Regional State, Southern Ethiopia.

The greenhouse pot experiment study was carried out at Hawassa University, School of Plant and Horticultural Sciences. An improved tomato (*Lycopersicon esculentum L.*) variety named Gelilema was used as a test crop with a seed rate of 150 g ha<sup>-1</sup>. A seed of a tomato variety Gelilema was obtained from Melkassa Agricultural Research Center (MARC) and was used for the experiments. Gelilema was released by MARC in 2015 and is still widely produced by smallholder farmers and is a high yielder (Sirba et al., 2022).

#### **1.4. Definition of core ideas and expressions used**

This section provides the definition of core ideas and the terminology used in this, arranged alphabetically as follows:

*Azolla*: indicates the *Azolla* is a genus of small, floating ferns that belong to the family Salviniaceae. These ferns are typically found in freshwater environments such as ponds, marshes, and rice fields.

*Nitrogen* is the primary source of macronutrients. It is vital because it is a major component of chlorophyll, the compound through which plants use sunlight energy to produce sugars from water and carbon dioxide (i.e., photosynthesis). It is also a major component of amino acids, the building blocks of proteins. Without proteins, plants wither and die.

*Nitrogen uptake*: it is the process through which plants absorb and assimilate nitrogen from their environment, mainly the soil, into their tissues.

*Physiochemical properties of soils*: this is a term that describes the combined effect of both the physical and chemical properties that define a soil's characteristics. Many of the physiochemical soil properties are partially defined by the percentage of sand, silt, and clay mineral particle sizes, bulk density, content, and the chemical properties of other soil parameters.

*Soil characteristics/properties:* Soil characteristics are single parameters that are observable or measurable in the field or laboratory. Soil properties are combinations of soil characteristics known to occur in soils and are considered indicative of present or past soil-forming processes.

*Soil characterization* is fundamental to all soils, as it is an important tool for soil classification, which is done based on soil properties. It also provides information for the understanding of the physical, chemical, mineralogical, and microbiological properties of the soils.

*Soil nitrogen (N) mineralization:* this describes that it is the biological conversion of organic nitrogen, found in soil organic matter, into inorganic forms, primarily ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), which are available for plant uptake. This process is crucial for plant nutrition, as it makes nitrogen, an essential nutrient, accessible for plant uptake and utilization.

*Soil nutrients* are the essential chemical elements found in soil that plants absorb to support their growth and development. They are the building blocks for plant tissues and play vital roles in various plant processes. These nutrients can be naturally present in the soil or added through fertilizers.

## **1.5. Structure of the Dissertation**

This PhD dissertation consists of four sections in the format of articles, preceded by a general introduction and description of the study areas. Each unit is considered as a separate chapter. This is followed by the general conclusion drawn from the entire study. Figures and tables are incorporated in the text, and appendices appear at the end. The dissertation includes the following chapters:

CHAPTER II: Characterization and classification of soils at Jara Gelalicha kebele (Hawassa Zuria district) and Aruma kebele (Wondo Genet district), Sidama region, and southern Ethiopia. This chapter focuses on the characterizing and classification of soils at the experimental sites of the Jara Gelalicha kebele (Hawassa Zuria) and Aruma kebele (Wondo Genet district). This chapter aims to characterize and classify the soils at the experimental sites, describe their morphological and physicochemical properties, identify the soil types, and classify the soils according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2022). One representative pedon from cultivated land was excavated at each location, and a total of twelve disturbed and twelve undisturbed soil samples (cores) were taken across the indicated

diagnostic horizon from both locations. Soil samples were examined in the lab to determine their physicochemical characteristics, and the results were then discussed in relation to soil fertility.

**Chapter III:** Influence of Azolla dry biomass and inorganic nitrogen fertilizer on soil chemical properties, yield and yield components of tomato under greenhouse conditions. The experiment was focused on soil chemical properties, as well as tomato growth and yield under greenhouse settings. The goal was to explore the effects of Azolla dry biomass and inorganic nitrogen on soil chemical characteristics, tomato yield, and yield components in a greenhouse pot study. Prior to treatment, composite soil samples were taken from both selected sites. Soil samples from each treatment were collected from both locations and analyzed after harvest.

**Chapter IV:** Enhancing soil fertility, nitrogen use efficiency and tomato productivity through integrated use of Azolla dry biomass and nitrogen fertilizers under irrigated conditions in Hawassa Zuria and wondo Genet, southern Ethiopia. The objective this chapter was designed to evaluate the integrated effects of Azolla dry biomass and inorganic nitrogen fertilizer on enhancing soil fertility, crop productivity and nitrogen use efficiency under irrigation conditions. Before planting, composite samples were collected from the two sites using an auger in a zigzag pattern. Post-harvest soil samples were collected from each treatment and analyzed for soil chemical properties

**Chapter V:** Effects of azolla dry biomass on soil nitrogen mineralization in soils collected from Hawassa Zuria and Wondo Genet districts incubated under laboratory conditions. The aims of this chapter was designed to determine the effects of Azolla dry biomass on soil nitrogen mineralization incubated under laboratory conditions and evaluates the maximum amount of nitrogen released during a period of incubation, as well as the amount of nitrogen mineralized during different incubation periods. For this study, soil samples were collected from a depth of 0-20 cm at two sites. Standard incubation procedures were followed, and the treatments were incubated for 56 days. The amount of inorganic nitrogen was measured at 7-day intervals.

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## **CHAPTER TWO**

### **CHARACTERIZATION AND CLASSIFICATION OF SOILS AT JARA GELALICHA AND ARUMA AREAS, SIDAMA REGION, SOUTHERN ETHIOPIA**

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## 2. CHARACTERIZATION AND CLASSIFICATION OF SOILS AT JARA GELALICHA AND ARUMA AREAS, SIDAMA REGION, SOUTHERN ETHIOPIA

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### Abstract

*Soil characterization and classification is a vital tool for classifying soil, identifying soil types, managing nutrients, and transferring research findings. Jara Gelalicha in the Hawassa Zuria district and Aruma in the Wondo Genet district of the Sidama Region require soil characterization and classification because of their unique soil properties, targeted research goals, environmental monitoring needs, land-use management considerations, and identify the soil fertility status. Therefore, this study was carried out in the specified locations to characterize the morphological and physicochemical properties as well as identify the types of soil in the studied areas. For this study, extensive preliminary site observations, reconnaissance surveys, and 30 auger observations were conducted before opening the profile pits in each location. One representative pedon was opened at each site to a depth of 2 x 2 x 2 m, and the profiles were described using the Guidelines for Field Soil Descriptions (FAO, 2006). A total of 12 disturbed and 12 undisturbed soil samples were collected from each diagnostic horizon of both locations. The soil analysis results showed that the surface horizon textural class of pedon of Jara Gelalicha was sandy loam; whereas, the textural class of the pedon at Aruma was sandy clay loam. In the Jara Gelalicha and Aruma locations, the OC content in the surface horizons were 2.39% and 3.57%, respectively. In the Jara Gelalicha and Aruma locations, the total nitrogen content of the surface soil horizons were 0.23% and 0.32%, respectively. Based on the World Reference Base for Soil Resources (WRB), the soils of the study areas were classified as Eutric Cambisols (Loamic) in Jara Gelalicha and Eutric Andosols (Loamic) in Aruma area. Therefore, the soil fertility management and land use decisions should consider such soil variability. The findings could offer crucial information for designing soil management options to increase soil productivity and to make appropriate land use decisions.*

**Keywords:** Morphological properties, soil horizons, soil profiles, soil properties, soil types

## 2.1. Introduction

Soils have various morphological, physical, chemical, and biological properties, leading to different reactions to management practices, abilities to provide ecosystem services naturally, resilience to disturbance, and susceptibility to deterioration (FAO, 2017). Therefore, it is crucial to characterize and classify soils to utilize these resources according to their capacities and manage them sustainably. Soil information gathered through systematic identification and grouping provides insight into the potential and limitations of the property, enabling efficient planning of various land uses (Lufega and Msanya, 2017). Soil characterization is essential for understanding the physical, chemical, mineralogical, and biological properties of soil, as well as for organizing knowledge and facilitating the transfer of experience and technology (Chekol and Mnalku, 2012; Adhanom and Teshome et al., 2016). According to Buol et al. (2003), soil classification is a vital tool for classifying soil, identifying soil types, managing property, and transferring research findings. To achieve the highest level of crop productivity, a comprehensive understanding of a nation's soil nature and characteristics is crucial for managing it according to its potential (Abayeneh and Berhanu, 2000). Soil characterization studies are fundamental for understanding, classifying, and gaining the best understanding of the environment (Onyekanne et al., 2012; Yitbarek et al., 2018). Soil categorization is critical for understanding soil behavior and establishes the foundation for effectively assessing soil suitability and maintaining fertility (Desalegn et al., 2014). However, previous soil assessments in Ethiopia were generally limited to certain areas (Yitbarek et al., 2018). As a result, many sections of the country continue to lack accurate soil information, despite its enormous size and wide range of soil types. In Ethiopia, the consideration of soil types has influenced agricultural production and methods for ecologically sound land management (Assen and Yilma, 2010). However, existing regional and small-scale studies on soil characterization and classification in Ethiopia have not adequately supplied essential soil data to support soil management practices tailored to local variations (Hailu et al., 2015; Mathwose and Mesfin, 2024).

In Ethiopia, most studies conducted so far have been at a small scale, which may not be applicable for site-specific land use and soil management. Therefore, comprehensive knowledge of soil characteristics at a large scale, local watershed, or farm level is essential for addressing specific and local problems in agricultural production (Hailu et al., 2015). These various soil

types were not fully characterized in the study locations, and their characteristics for soil fertility purposes were not well characterized. Because diverse soil types have varied physicochemical and morphological properties, alternative farming methods and inputs have been proposed in different parts of Ethiopia (Getahun, 2015). Soil types and properties in Ethiopia vary significantly due to factors such as climate, topography, parent material, and vegetation, which vary in their influence across regions (Chemedda et al., 2017; Mengiste et al., 2025). This diversity emphasizes the importance of soil classification as a instrument for facilitating technology transfer and information exchange across stakeholders (Fekadu et al., 2018). Despite these needs, Ethiopia's soil information database remains limited due to insufficient survey activities, attributed to the country's expansive area and complex topography (Mulugeta and Sheleme, 2010).

Sustainable soil usage needs an accurate understanding of soil composition and fertility control. These characteristics provide critical information about soil type, fertility, and productivity, all of which are important for sustaining livelihoods, ensuring food security, and decreasing the risk of malnutrition (Uwitonze et al., 2016; Mohamed et al., 202; Merumba et al., 2024). Obtaining information about soils requires classifying and categorizing them because of their diverse morphological, physical, chemical, and biological characteristics (Mathewos and Mesfin, 2024). In order to identify the chemical, physical, and mineralogical characteristics of soil, a scientific method for collecting soil data is soil categorization (Sharu et al., 2013). The Sidama region's soils are classified into major Reference Soil Groups (RSG), which include Luvisols, Cambisols, Nitisols, Vertisols, Andosols, Fluvisols, Regosols, and Leptosols (Bizunhe, 2023).

In the Sidama region, specifically in the study areas of Jara Gelalicha in Hawassa Zuria and Aruma in Wondo Genet districts, there is a problem of lack information on soil characterization and classification. The soil type remains unknown, leading to a lack of knowledge among agricultural experts regarding the available and deficient nutrients in the soil. Information on the morphological and physicochemical characteristics of the soil, as well as its fertility status, is inadequate. Previous soil resource studies in the national level have been conducted at small scales with a high level of generalization. However, the soils of Aruma in the Wondo Genete district and Jara Gelalicha in the Hawassa Zuria district of the Sidama region have yet to be characterized or classified. In the study areas agricultural field experiments have been carried out, which need soil information to interpret results and recommend appropriate soil management

strategies for optimum and sustainable agricultural production. Generally, the current soil information of the country is not exhaustive to support appropriate land use decision and sustainable land management strategies for every area of the country. Therefore, the aim of the study was to characterize and classify soils of Jara Gelalicha and Aruma areas of Sidama Region, Southern Ethiopia

## **2.2. Materials and Methods**

### **2.2.1 Description of study areas**

#### **2.2.1.1. Location and climate of the study areas**

The study was conducted in farmers' fields in Jara Gelalicha Kebele in Hawassa Zuria district and Aruma Kebele in Wondo Genet district of the Sidama Regional State, Southern Ethiopia. Jara Galalicha Kebele is located 290 km south of Addis Ababa and 16 km from Hawassa town. Geographically, the area lies at 7°0'13.7" to 7°48'11.5" N and 38°21'21.1" to 38°63'30.5" E, with a mean altitude of 1712 meters above sea level (masl) and slope ranges from 0.2- 0.5% (gentle). It receives a mean annual rainfall of 952 mm with mean minimum and maximum temperatures of 12.1°C and 26.7°C, respectively (Hawassa Meteorological Center, 2018). The soil texture was sandy clay loam. Based on the IUSS WRB(2022) The dominant soil types was classified as Cambisols. The major crops grown in the study area are maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* L.), wheat (*Triticum aestivum* L.), enset (*Ensete ventricosum* (Welw.), beetroot (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), sweet potato (*Ipomoea batata* L.), khat (*Catha edulis* L.), and chili (*Capsicum annum* L.).

Aruma Kebele, in the Wondo Genet district is located 264 km south of Addis Ababa and 27 km southwest of Hawassa town. Geographically, the area is located at 6°16' to 7°45' N and 38°31' to 38°63' E. The altitude of the district ranges from 1001 to 1780 meters above sea level. The mean annual rainfall in the district ranges from 801 to 1221 mm, and the mean annual temperature ranges from 17.6 to 22.5°C (SNNPRS, BoFED, 2018). The soil texture was sandy loam. Based on the IUSS WRB(2022) the dominant soil types was classified as Andosols. The major crops grown in the study area are khat (*Catha edulis* L.), sugarcane (*Saccharum officinarum* L.), maize (*Zea mays* L.), enset (*Ensete ventricosum* (Welw.) Cheesman), cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* L.), and potato (*Solanum tuberosum* L.). Both locations exhibit

a landscape characterized by a mix of agricultural land, natural vegetation, and forest cover. The soil pedons of the experimental sites were excavated in the cultivated land. In both locations, the natural vegetation cover prior to cultivation was forest. The map of the study areas is presented in Figure 2.1. The ten-year annual climate data (2013-2022) for Hawassa Zuria district and Wondo Genet district are presented in Figures 2.2 and 2.3, respectively.

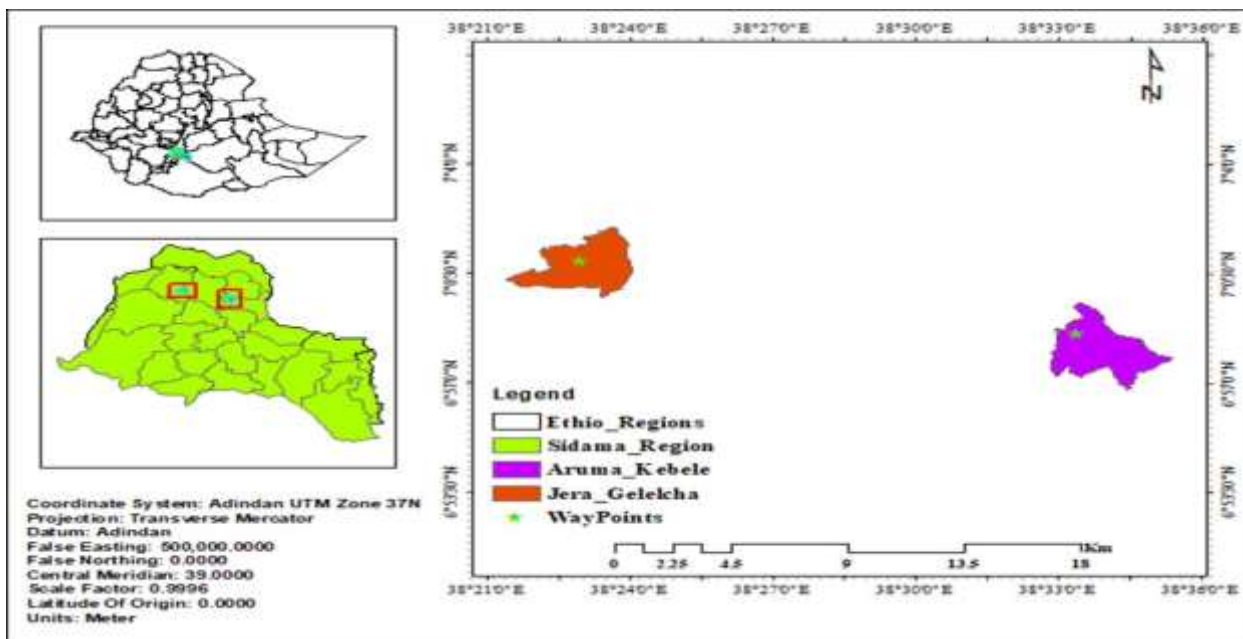


Figure 2.1. Location map of study areas

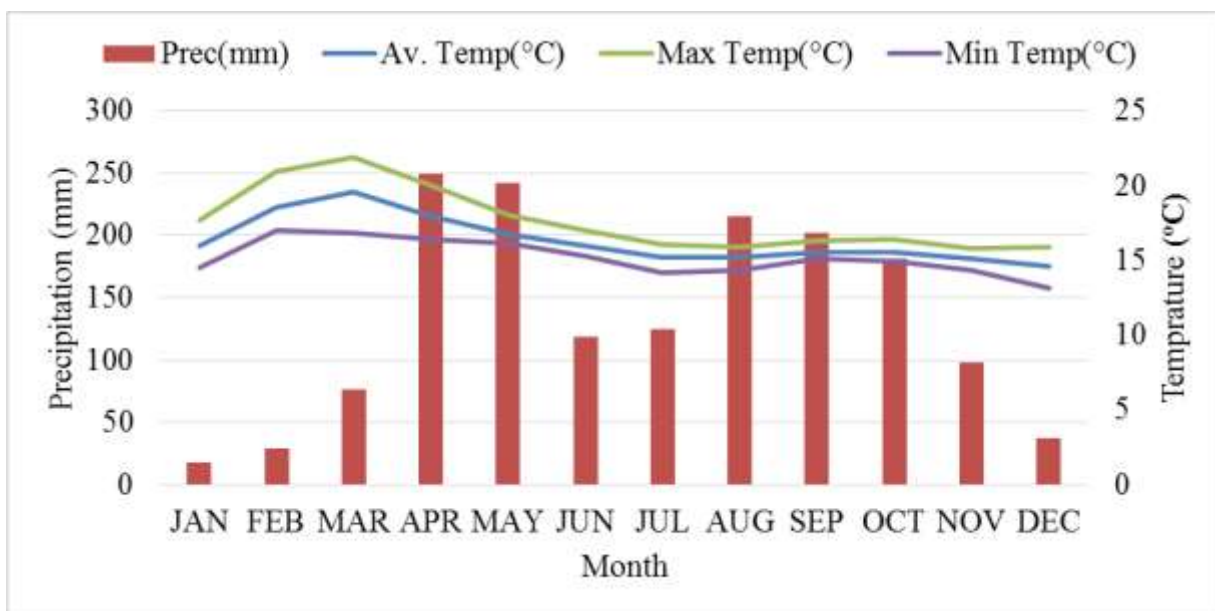


Figure 2. 2. Annual Climate data for the Hawassa Zuria district (2013-2022) (Source: Hawassa Meteorology Station)

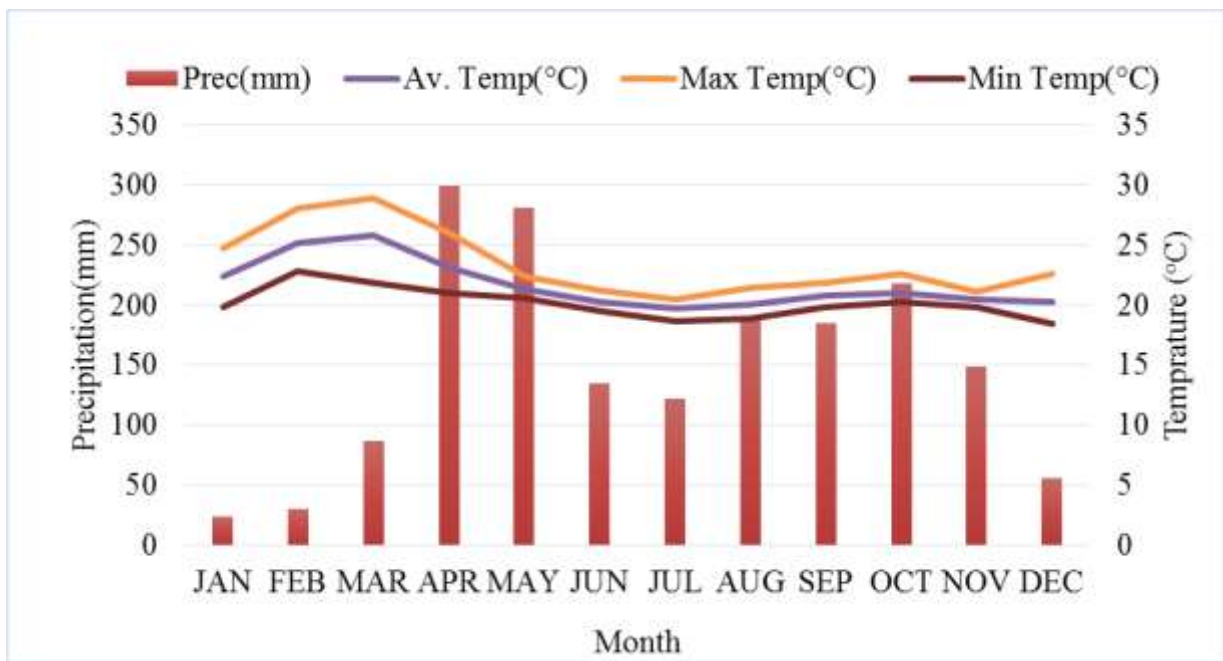


Figure 2. 3. Annual climate data for the Wondo Genet (2013–2022 average) (Source: Hawassa Meteorology Station)

#### 2.2.1.2. The geology and geomorphology of the study areas

The Hawassa area is morphologically part of the rift floor zone (Bizunhe, 2023). It is a depression that was created by volcano-tectonic collapse phenomena (Berhanu et al., 1999). According to Ayele et al. (2022), the Hawassa area contains fluvial sediments, alluvial sediments, Wondo Koshe pumice fall and flow deposits, scoria cones, tuff cones, Hawassa basalts, and Hawassa rhyolitic ignimbrites. According to Temesgen et al. (1999), the Wondo Genet area is characterized by tertiary and quaternary geological formations. The area mainly consists of ignimbrites, pumiceous tuff, and volcano-lacustrine deposits interbedded with basaltic lava.

#### 2.2.2. Field survey, soil profile selection, and soil sample preparation

A preliminary site and auger observation was carried out prior to opening the pedons. For each study area, thirty auger inspections were made, and a representative site was selected. In each study area, a representative pedon of 2 x 2 x 2 meters in length, width, and depth was opened and described based on guidelines for soil description (FAO, 2006). General site information was recorded based on the standard profile description sheet. From each identified horizon, about 1 kg of soil samples were taken with plastic bags and delivered to Hawassa University's Soil Science laboratory for soil physical and chemical parameter analysis. The Munsell Color Chart (KIC, 2000) was used to identify the color of each horizon (both moist and dry).

### 2.2.3. Soil Analysis

The physical and chemical properties that were considered for the study were texture, bulk density, particle density, total porosity, soil water retention capacity at field capacity (FC) and permanent wilting point (PWP), available water content (AWC), pH, electric conductivity, organic matter, total nitrogen, phosphorus, CEC, exchangeable bases, and micronutrients. Particle size distribution was analyzed using the hydrometer method (Bouyoucos, 1962). Bulk density was calculated from undisturbed soil samples collected with a core sampler, weighed for field moisture content, and dried in an oven at 105°C, following the method described by Sahlemedhin and Taye (2000). Particle density was determined using the pycnometer method (Blake, 1965). Total porosity was calculated from the bulk density and particle density values. The bulk density was calculated by dividing the mass of the oven-dried soils by their natural volumes in the field. Bulk density (BD) and particle density (PD) were used to calculate total porosity, which was as follows Equation: Total porosity (%) = [1-(BD/PD)] x100 ----- (1)

The water content of each soil horizon was measured at field capacity (FC) at -1/3 bar and permanent wilting point (PWP) at -15 bars using the Ktues (1965) pressure plate apparatus. Hillel (1980) calculated the soil's available water-holding capacity by subtracting the PWP water content from the FC water content. The depth of the available water content (AWC; mm m<sup>-1</sup>) was determined using Equation 2:

$$AWC = 1000 \times \left( \frac{FC - PWP}{100} \right) AS \text{ ----- (2)}$$

Where AWC is the available water content (mm m<sup>-1</sup>), FC is the gravimetric water content in field capacity (% weight), PWP is the gravimetric water content at the permanent wilting point (% weight), and AS is the apparent specific gravity (the ratio of soil BD to the density of water) (Asawa, 2006). Soil pH was measured in a 1:2.5 soil-to-water suspension water mixture using a pH meter (Van Reeuwijk, 1992). Electrical conductivity (EC) was measured using a conductivity meter in a soil–water extract (Okalebo et al., 2002). Soil organic carbon was determined using the Walkley and Black wet oxidation method (Walkley and Black, 1934). Total nitrogen was determined using the Kjeldahl procedure (Wilke, 2005). Available soil phosphorus (P) was analyzed using the standard procedure of the Olsen et al. (1954) extraction method. The cation

exchange capacity was determined following the ammonium acetate method (Sarkar and Haldar, 2005). From the aliquots of the same extract, exchangeable bases were extracted with 1M ammonium acetate at pH 7 (Sahlemeden and Taye, 2000). Exchangeable Ca and Mg were measured from the extract using an atomic absorption spectrophotometer (AAS), while exchangeable K and Na were determined from the same extracts using a flame photometer, as described by Rowell (1994). Percent base saturation (PBS) was calculated as the percentage of total exchangeable bases to cation exchange capacity (CEC) of the soil. Calcium carbonate content was determined following the acid neutralization method in which the soil carbonate was decomposed by excess standard HCl solution and back-titrated with standard NaOH after filtering it (Jackson, 1958). Available Fe, Mn, Zn, and Cu were extracted from the soil samples with diethylene triamine pentaacetic acid (DTPA) as described by Lindsay and Norvell (1978), and the contents in the leachate were measured using an atomic absorption spectrophotometer (AAS).

#### **2.2.4. Soil Classification**

A preliminary soil classification was carried out in the field using field analysis of site features and profile descriptions. The soils were finally classified into two major soils units based on their morphological, physical and chemical properties according to the IUSS Working Group WRB, (2022).

#### **2.2.5. Statistical Analysis**

Data obtained from physical and chemical analysis were analyzed using the general linear model procedure of the Statistical Analysis System (SAS, 2016) for the Pearson correlation analysis of soil properties in both study areas.

## 2.3. Results and Discussion

### 2.3.1. Morphological and physical properties of the soil in the study areas

#### 2.3.1.1. Morphological properties

The site characteristics and physiographic settings of pedons in the study sites was summarized in Table 2.1, which were found to be very deep (>200 cm) in both locations. According to Moshago et al. (2022) and Mengiste et al. (2025), the depth of the soil affects root development as well as water and nutrient storage in plants. Table 2.2 provides a summary of the profiles' morphological features. The full morphological descriptions are reported in the appendices (Appendix 2.1 and Appendix Table 2.2). While the subsurface ranged in color from reddish brown (5YR4/3) to light brown (7.5YR6/3) and dark brown (7.5YR3/2) to brown (7.5YR4/4), the moist color of the surface horizons was dark gray (5YR4/1) and very dusky red (2.5YR2.5/2). The Aruma area showed a darker surface horizon compared to Jara Gelalicha, possibly due to the accumulation of high organic matter. Color variations within a soil profile and between two pedons are most likely caused by differences in parent material types, drainage conditions, organic matter concentration, and iron oxide forms (Abate et al., 2014; Alemu and Buraka, 2018; Dinssa and Elias, 2021; Mathwos and Mesfin, 2024). Mulugeta and Sheleme (2010) found similar observations, in which the surface horizons were darker than the subsurface horizons, due mostly to organic material buildup and breakdown.

Similar findings were reported by Mulugeta and Sheleme (2010), where the surface horizons were darker compared to the subsurface horizons, mainly due to the accumulation and decomposition of organic materials. Soil color variations in pedons may be due to variations in clay, organic matter content, parent material, and drainage conditions, which influence redoximorphic reactions. This study aligns with Hailu et al.'s (2015) findings on soil color. The surface layers in both pedons were darker in color than the underlying horizons (Table 2.2). Furthermore, compared to Jara Gelalicha, the surface soil of Aruma pedon had a comparatively dark and moist color; this could be due to the higher content of organic matter (Alem et al., 2015).

Table 2.1 The site characteristics and physiographic settings of the pedons in the study areas.

Areas	Topographi	Location	Slope	Altitude	Surroundin	Parent
-------	------------	----------	-------	----------	------------	--------

c position		Latitude	Longitude	(%)	(masl)	g landform	material s
Jara Gelalich a		7°0'29.2 ''N	38°23'35. 9''E	0.2	1701	L	Alluvial
Aruma	Upper	6°58'2.8 ''N	38°33'55. 5''E	0.2	1756	P	Volcani c ash

L=level; P=plain

Table 2.2 Selected morphological characteristics of cultivated soils of Jara Gelalicha and Aruma areas

Pedo n	Depth(c m)	Horizo n	Color		Structure grade/size/ty pe	Consistency			BO U
			Moist	Dry		Dry	Mois t	Wet	
Jara Gelalicha									
1	0-18	Ap	5YR4/1	2.5Y6/1	MO,F,GR	SH A	FR	SS,SP	C,S
	18-40	AB	5YR4/3	2.5Y7/1	MO,M,AB	SH A	FR	SS,SP	C,S
	40-80	Bw	2.5YR4/3	7.5YR8/1	MO,M,AB	SH A	VFI	SS,SP	D,S
	80-110	BC1	5YR5/4	2.5Y7/1	WE,M,AB	SH A	VFI	SS,SP	D,S
	110-160	BC2	7.5YR5/4	10YR7/1	WE,M,SG	SH A	EFI	SS,SP	C,W
	160-200 <sup>+</sup>	C	7.5YR6/3	2.5Y6/4	WE,M,MA	SH A	EFI	NS,N P	----- -
Aruma									
	0-20	Ap	7.5YR2.5 /2	7.5YR2.5 /4	WE,M,GR	SH A	FR	S,P	C,S
1	20-70	AB	7.5YR3/2	7.5YR3/1	WE,M,SAB	HA	FR	VS,V P	D,S
	70-115	B1	7.5YR3/1	7.5YR4/1	WE,M, AB	HA	FR	VS,V P	D,S
	115-140	B2	7.5YR3/4	7.5YR5/2	WE,M,SAB	VH A	VFR	VS,V P	A,S
	140-164	2BC	7.5YR4/4	7.5YR5/2	WE,F,SG	VH A	VFR	SS,SP	C,S
	164-200 <sup>+</sup>	2C	7.5YR4/4	7.5YR4/2	WE,F,SG	SO	LO	NS,N P	-----

BOU= boundary; WE= weak; MO= moderate; VM= very fine to medium; VF= very fine; FC= fine to coarse; SG= single grain; GR= granular; AB= angular blocky; SAB= subangular blocky;

MA= massive; FR= friable; SHA= slightly hard; HA= hard; VHA= very hard; SO= soft; Lo= loose; F= friable; VFR= very friable; VST= very sticky; SST= slightly sticky; ST= sticky; PL= plastic; VPL= very plastic; SPL= slightly plastic; A= abrupt; D= diffuse; C= clear; S= smooth; W= wavy.

### **2.3.2. Physical properties of soils in the study areas**

#### **2.3.2.1. Particle size distribution, bulk density, total porosity, and soil moisture content**

The proportion of sand was highest in both profile horizons at the Jara Gelalicha and Aruma locations. The sand and silt content of the Jara Gelalicha pedon was relatively higher than the Aruma pedon (Table 2.3). However, the clay content of Aruma pedon was relatively higher. The surface horizon textural of pedon of Jara Gelalicha was sandy loam; whereas, the textural class of the pedon at Aruma was sandy clay loam. Regarding the subsurface horizons, most of the textural class of the horizons at Jara Gelalicha pedon was sandy loam. In the case of the Aruma pedon, most of the subsurface horizons textural class was sandy clay loam. Clay, sand, and silt percentages were classified by Hazelton and Murphy (2016) as very high, high, moderate, low, and very low categories, respectively, >50, 40–50, 25–40, 10–25, and <10. According to these classifications, the Jara Gelalicha soils had sand content ranging from very high to high, the silt content ranged from moderate to low, and the clay content ranged from low to very low (Table 2.3). In the Aruma soils, sand content had ranged from very high to high, silt content was low, and clay content had ranged from moderate to low, respectively. The sand concentrations of both soil profiles reveal an unsystematic trend with increasing depth. Similar findings were published by Sebnie et al. (2021), who found that the amount of sand increased inconsistently with depth. Soils with a silt/clay ratio greater than 0.15 are relatively younger and have a higher intensity of weathering potential. This indicator is crucial for assessing soil weathering rates and determining a specific soil profile's relative growth stage (Debele et al., 2018; Mathwos and Mesfin, 2024). Additionally, a similar result was reported by Klomit et al. (2000). Soils with a silt/clay ratio of 0.15 are regarded as highly weathered. Young parent materials usually have a silt/clay ratio above 0.15 (Sharu et al., 2013). These results indicate that the soils in Jara Gelalicha are relatively young and have a high degree of weathering potential. Similar results have been reported for other soils in similar ecological settings (Kebeney et al., 2014; Kalala et al., 2017; Hundessa, 2020; Walche et al., 2023). Additionally, Giday et al. (2015) reported a similar result in Southern Tigray, finding that silt-to-clay ratios were greater than 0.38. The silt-to-clay ratio in the soil of the Jara Gelalicha site was higher compared to the Aruma site, indicating that the soils are relatively young with a high degree of weathering potential (Asomoa, 1973). This could be due to

the volcanic parent materials surrounding Hawassa, consisting of alkaline lavas, ash, and ignimbrites, primarily from tertiary and younger ages (Meron, 2007), which can lead to the formation of young soils with a coarser texture.

In the Jara Gelalicha and Aruma locations, the bulk densities of the surface horizon were 0.89 g/cm<sup>3</sup> and 0.87 g/cm<sup>3</sup>, respectively (Table 2.3), whereas the bulk densities of the subsurface horizons ranged from 0.92 to 1.13 g/cm<sup>3</sup> and 0.90 to 1.67 g/cm<sup>3</sup>, respectively (Table 2.3). According to Hazelton and Murphy (2007), bulk density (BD) values <1, 1-1.30, 1.30-1.60, 1.60-1.90, and >1.90 g/cm<sup>3</sup> are classified as very low, low, medium, high, and very high, respectively. The study supports Brady and Weil's (2008) and Sebnie et al. (2021) findings that soil bulk density increases with soil depth. Higher soil bulk densities in subsurface horizons are due to higher soil organic matter content compared to surface horizons (Wakene, 2001; Achalu et al., 2012; Abera et al., 2016). Soils with low bulk density exhibit favorable soil physical conditions, whereas, high bulk density exhibit poor soil physical conditions (Hajabbasi et

al., 1997; Patil and Prasad, 2004). The lower bulk density in the surface horizon may be due to cultivation, high organic matter content, and biotic activities (Sekhar et al., 2014).

The total porosity (TP) of the surface horizons of Jara Gelalicha and Aruma soils was 66.16% and 67.29%, respectively. The total porosity of the subsurface horizons ranged from 48.87% to 65% and 33% to 65.52%, respectively (Table 2.3). Therefore, the total porosity typically falls within the range of 30% to 70% (Hazelton and Murphy, 2007). However, it decreased consistently with soil depth in both areas of the pedons. This decrease could be the decrease in soil organic matter down the soil depth. The pressure of the overlaying horizons could also have an impact on the porosity of the soils. These findings are consistent with Hailu et al. (2015), who also observed a decrease in total porosity with increasing soil depth due to compaction, reduced rooting effects, and lower organic matter content.

The surface soils of Jara Gelalicha and Aruma had available water content (AWC) values of 113.34 mm m<sup>-1</sup> and 200.10 mm m<sup>-1</sup>, respectively. In contrast, the subsurface soils had values ranging from 66 mm m<sup>-1</sup> to 100.28 mm m<sup>-1</sup> and 149.65 mm m<sup>-1</sup> to 196.00 mm m<sup>-1</sup> (Table 2.3). As soil depth increases, its ability to retain moisture decreases. This is because topsoil contains more soil organic matter than subsoil (Achalal et al., 2012), which increases the porosity of the soils. Sebnie et al. (2021) discovered similar findings, demonstrating that surface horizons had a greater amount of available water than subsurface horizons.

Table 2.3 Selected physical characteristics of soil horizons in pedon at study areas

Horizon	Depth(cm)	Particle size distribution (%)			Textural class	S:C	BD PD		TP (%)	FC (v/v %)	PWP (v/v %)	AWC(mm m <sup>-1</sup> )
		Sand	Silt	Clay			(g cm <sup>-3</sup> )					
Jara Gelalicha												
Ap	0-18	58	29	13	SL	2.23	0.89	2.63	66.16	33.5	21.18	113.34
AB	18-40	50	29	21	SCL	1.26	0.92	2.60	65.00	35.48	24.51	100.28
Bw	40-80	52	29	19	SL	1.53	0.94	2.42	61.15	28.34	17.14	105.28
BC1	80-110	52	34	15	SL	2.27	0.98	2.35	58.30	29.48	19.95	93.39
BC2	110-160	72	17	11	SL	1.55	1.00	2.24	55.36	31.10	24.50	66.00
C	160-200 <sup>+</sup>	68	23	9	SL	2.56	1.13	2.21	48.87	23.10	17.05	68.40
Aruma												
Ap	0-20	52	15	33	SCL	0.45	0.87	2.66	67.29	43.00	20.00	200.10
AB	20-70	44	21	35	CL	0.60	0.90	2.61	65.52	39.00	22.00	190.21
B1	70-115	46	17	37	SC	0.46	1.40	2.58	46.00	40.00	26.00	196.00
B2	115-140	52	14	34	SCL	0.42	1.60	2.56	38.00	32.42	20.35	193.12
2BC	140-164	50	22	28	SCL	0.78	1.65	2.53	35.00	32.42	23.35	149.65
2C	164-200 <sup>+</sup>	56	22	22	SCL	1.00	1.67	2.49	33.00	39.12	29.23	165.16

Note: CL= loam clay, SC= sandy clay, SCL= sandy clay loam, SL= sandy loam BD = bulk density, PD = particle density, TP = total porosity, S: C = sand to clay ratio, FC = field capacity, PWP = permanent wilting point, AWC = available water content

### **2.3.3. Chemical properties of soils in the study areas**

#### **2.3.3.1. Soil pH and electrical conductivity**

In the Jara Gelalicha and Aruma locations, the surface soil pH (H<sub>2</sub>O) values were 7.7 and 7.1, respectively (Table 2.4), while the subsurface values varied from 8.2 to 9.3 and 7.3 to 7.6, respectively (Table 2.4). Both profiles mostly showed regular variations with depth in pedons, with an increasing trend observed in both locations. Soil reaction generally revealed an increasing trend throughout the soil depth in the pits. At each site, the surface soils had the lowest pH values, while the subsurface soils had greater pH values (Sharu et al., 2013; Yitbarek et al., 2018; Mathewos and Mesfine, 2024). According to Ayalew and Beyene (2012), lower organic matter content with depth results in less H<sup>+</sup> ion discharge from the breakdown of organic matter, and a rise in soil pH along soil depth may suggest the existence of vertical explorations of exchangeable bases. Similar findings were reported by Senbie et al. (2021), confirming that an increase in soil pH with depth could indicate leaching of exchangeable basic cations, and the decomposition of organic matter releases fewer H<sup>+</sup> ions as the organic matter content decreases with depth.

In the Jara Gelalicha and Aruma locations, the electrical conductivity (EC) of the surface horizon was 2.58 dS/m and 1.42 dS/m, respectively (Table 2.4). The subsurface horizons ranged from 2.86 to 4.66 dS/m and 0.62 to 1.53 dS/m, respectively (Table 4). Generally, in the Jara Gelalicha study area, the electrical conductivity of the soil horizons increased with depth. The soils in the Aruma area had very low electrical conductivity values ranging from 0.62 to 1.53 dS/m, indicating non-saline soils (Debele et al., 2018). Electrical conductivity content in the Aruma area showed irregular patterns with increasing depth in parts of the pedon horizon, indicating a low level of leaching in the area. Both the surface and subsurface EC values of the Aruma soils are less than 2 dS/m, indicating that they are not saline. Electrical conductivity measurements in the soils under investigation show that the soluble salt content is below the thresholds where soil salinity has no negative effects on crop development and productivity (Mathewos and Mesfin, 2024 and Sebnie et al., 2021).

According to Lal and Shukla (2004), the soil's electrical conductivity in the research area was less than 2 dS/m, indicating no decrease in yield. The electrical conductivity values measured in Jara Gelalicha soils revealed that soluble salt concentrations are below the threshold at which soil

salinity affects crop growth and yield (United States Salinity Laboratory Staff, 1954). The soils in the Jara Gelalicha location had alkaline reactions with a pH of over 7.7 and electrical conductivity (EC) of >2.58 dS/m, similar to results reported by Ayelew and Beyene (2012).

### **2.3.3.2. Organic carbon, total nitrogen, carbon to nitrogen ratio, available phosphorus, and calcium carbonate content**

In the Jara Gelalicha and Aruma locations, the organic carbon (OC) content in the surface horizons was 2.39% and 3.57%, respectively (Table 2.4). In the subsurface horizons, it varied from 0.97% to 1.85% and 1.46% to 2.76%, respectively (Table 2.4). Maria and Yost (2006) classified organic carbon content of <1.5%, 1.5-2.5%, and >2.5% as low, medium, and high, respectively. The high organic carbon content in the surface soil was attributed to the significant amount of rainfall that promotes high biomass production, resulting in a high amount of organic matter in the soil. However, the profiles of both soils revealed that the OC content declined with soil depth, indicating that top soils accumulate more organic materials (Sebnie et al., 2021; Mathwos and Mesfin, 2024). There have been reports of low levels of organic carbon in several parts of Ethiopia (Beyene, 2017; Zewide et al., 2018; Mathwos and Mesfin, 2024; Mengiste et al., 2025). Surface horizons have a higher addition of decomposable organic materials and higher levels of organic carbon, according to Dinssa and Elias (2021). Additionally, comparable findings were reported by Ashokkumarid and Jagdish (2010) and Sekhar et al. (2017), who discovered that higher organic matter in surface soils was attributable to continual addition of organic matter from leaf fall, stubble, roots, and organic manure applied solely to the surface layers.

In the Jara Gelalicha and Aruma locations, the total nitrogen content of the surface soil horizons was 0.23% and 0.32%, respectively (Table 2.4). In the subsurface horizons, the range was from 0.10% to 0.19% and 0.15% to 0.27%, respectively (Table 2.4). Similar to organic carbon, total nitrogen content decreased with depth in both pedons (Table 2.4). The Landons (1991) rating system was used to categorize total nitrogen as low (0.2), medium (0.2-0.5), and high (0.5). The amount of total nitrogen varied within and between profiles depending on the amount of organic matter present. The surface horizon had a high total nitrogen content that gradually decreased through the profiles of Jara Gelalicha and Aruma. Compared to the Aruma area soils, the Jara Gelalicha soil contained less total surface nitrogen. These findings are consistent with those of Yitbarek et al. (2018) and Demiss and Beyene (2010), who found that the total nitrogen content

at their study sites decreased with depth. Additionally, similar results were reported by Dash et al. (2019), Dorji et al. (2014), Kumar et al. (2012), and Khanday et al. (2018). A regular trend of decreasing organic carbon with depth was observed in the soil profiles. According to Hartz (2007), soils with less than 0.07% total nitrogen have limited nitrogen mineralization potential, while those with greater than 0.15% total nitrogen would be expected to mineralize a significant amount of nitrogen during the succeeding crop cycle, indicating that most of the soils have good potential for nitrogen mineralization. In the Jara Gelalicha and Aruma areas, the Pearson correlation also revealed a strong and significant positive correlation ( $r = 0.892^{***}$  and  $0.981^{***}$ ,  $P \leq 0.001$ ) between total nitrogen (N) and organic carbon (OC) contents, respectively. This indicates that organic carbon is the principal source of nitrogen in the soil. These results in line with the findings of Mengiste et al. (2025), who found that TN concentration dropped as profile depth increased. .

Surface horizons of the C/N were 10.39 and 11.15 in the Jara Gelalicha and Aruma areas, respectively, while in the subsurface horizons, it ranged from 9.50 to 10.10 and 9.74 to 10.23 in the Jara Gelalicha and Aruma areas, respectively (Table 2.4). In comparison to their underlying horizons, the C/N ratio values of both studied pedon surfaces were consistently higher as soil depth increased. In both locations, the C/N ratio in the surface horizons is generally larger than 10:1, which implies a considerably better decomposition rate, improved nitrogen availability to plants, and potential for incorporating agricultural leftovers into the soil (Lelago and Buraka, 2019).

Surface horizons of the carbon to nitrogen ratio is an indicator of nutrient immobilization and mineralization, whereby a low C/N ratio indicates a higher mineralization rate and a higher C/N ratio indicates a higher immobilization rate (Anbessa, 2018). In general, a C/N ratio of about 10:1 suggests a comparatively better decomposition rate that serves as an index of enhanced N availability to plants and the possibility of incorporating crop residues into the soil without having any adverse effect on N immobilization (Assen and Yilma, 2010). Accordingly, the C/N ratio of the surface soils in all areas was not far from the optimum range in all soils for microbial needs. The C/N ratio of the soils showed a decreasing trend with increasing soil depth in both areas of the soil profiles. Achalu et al. (2012) found a narrow C/ N ratio in cultivated land soils due to increased organic carbon mineralization and temperature-induced aeration during tillage.

In the Jara Gelalicha and Aruma areas, the available phosphorus content in the surface horizons was 9.58 mg kg<sup>-1</sup> and 16.35 mg kg<sup>-1</sup>, respectively (Table 2.4). According to Ayalew (2016), surface soils in some southern Ethiopian locations contained a moderate amount of available phosphorus (5–15 mg kg<sup>-1</sup>). In the subsurface horizons, available P contents ranged from 1.22 to 7.20 mg kg<sup>-1</sup> and 2.10 to 11.29 mg kg<sup>-1</sup>, respectively (Table 2.4), which could be classified as very low to medium (Hazelton and Murphy, 2007; Jones, 2003). The authors classified Hazelton and Murphy (2007) classified soils with phosphorus values less than 5, 5–9, 10–17, 18–25, and <25 mg kg<sup>-1</sup> as very low, low, medium, high, and very high, respectively. The available P content of the soils decreased as they moved down the profile. Sekhar et al. (2017) reported similar results, finding that surface horizons had the highest accessible phosphorus levels, which decreased with depth. This may be due to crop cultivation being confined to the rhizosphere, leading to the need for external fertilizers to restore depleted phosphorus levels. Higher available P values in the surface horizon compared to subsurface horizons may be attributed to differences in the OM content and optimum soil pH.

According to Carrow et al. (2004), P-Olsen levels between 12 to 18 mg kg<sup>-1</sup> are considered sufficient, indicating that the available P in the surface horizons of Jara Gelalicha falls within the insufficient range. Available P content decreased with increasing depth, likely due to a decrease in soil organic matter. In high pH conditions where calcium is the dominant cation, phosphate tends to precipitate with calcium (Rishid, 2019). The soil in these locations was saline, with a pH consistently above 8. Due to the high pH of saline soil, more than 75% of phosphorus fertilizer combined with calcium in the soil, forming phosphates with low solubility. This resulted in reduced phosphorus availability and utilization of phosphorus fertilizer (Liu et al., 2024). Soil pH is a crucial factor that influences the occurrence and transformation of phosphorus forms in soil, affecting processes such as chemical fixation and precipitation dissolution of phosphorus in soil (Zhang et al., 2014). This finding is consistent with Debele et al. (2018) in the Muger sub-watershed, who found that the available phosphorus ranged from medium to high. Due to the decreasing soil organic matter content, the available phosphorus showed a declining trend for all soil profiles throughout the soil depth (Ayalew et al., 2015; Debele et al., 2018). This conclusion aligns with that of Ali et al. (2010), who discovered that the surface horizon had the highest concentration of available phosphorus in the soil.

In the Jara Gelalicha and Aruma locations, the calcium carbonate ( $\text{CaCO}_3$ ) content with in pedons varied from 2.07% to 2.36% and 0.23% to 0.61%, respectively (Table 2.4). In the Jara Gelalicha pedon, the increase can be attributed to the movement of calcium carbonate solutes downwards from the surface horizon to the subsurface horizon and soil parent material As a result; there was a visible presence of calcaric soil material in the Jara Gelalicha profile. Generally, the results revealed that all layers of the profiles had greater than 2%  $\text{CaCO}_3$ , resulting in the absence of calcaric soil material (Hazelton, 2007). In Aruma, the amount of  $\text{CaCO}_3$  contents showed an irregular pattern in the soil horizons with increasing depth, indicating a low degree of leaching process in the area. In the Aruma soil profile, the amount of  $\text{CaCO}_3\%$  showed no visible presence of calcaric soil materials at any horizons, as the results showed that all horizons in the profile were less than 2%, resulting in the absence of calcaric soil material (Hazelton, 2007).

Table 2.4. Selected chemical properties of Jara Gelalicha and Aruma area soils

Profile Horizon	pH( $\text{H}_2\text{O}$ )	EC(dS/m)	OC %	TN %	C:N	Ava. P(mg $\text{kg}^{-1}$ )	$\text{CaCO}_3$ (%)
Jara Gelalicha							
Ap	7.7	2.58	2.39	0.23	10.39	9.58	2.07
AB	8.2	2.86	1.85	0.19	9.74	7.20	2.11
Bw	8.8	2.87	1.52	0.16	9.50	2.90	2.17
BC1	9.1	3.95	1.36	0.14	9.72	1.97	2.19
BC2	9.3	4.40	1.01	0.10	10.10	1.32	2.24
C	9.3	4.66	0.97	0.10	9.80	1.22	2.36
Aruma							
Ap	7.1	1.42	3.57	0.32	11.15	16.35	0.23
AB	7.3	1.53	2.76	0.27	10.23	11.29	0.37
B1	7.4	1.10	2.39	0.24	10.00	9.84	0.32
B2	7.4	0.66	2.09	0.21	10.43	4.32	0.49
2BC	7.6	0.62	1.68	0.17	9.88	2.20	0.63
2C	7.5	1.53	1.46	0.15	9.74	2.10	0.59

Note: EC = electrical conductivity; TN = total nitrogen; C: N = carbon to nitrogen ratio; Ava. P = available phosphorus.

### 2.3.3.3. Cations exchange capacity, Exchangeable bases, Percent base saturation, and total exchangeable base of Soils

The CEC values of the surface horizon at Aruma and Jara Gelalicha were 24.08  $\text{cmol}(+)/\text{kg}$  and 15.83  $\text{cmol}(+)/\text{kg}$ , respectively (Table 2.5). The subsurface horizons had CEC values ranging

from 13.07 to 39.32 cmol (+)/kg and 23.12 to 27.14 cmol(+)/kg, respectively (Table 2.5). The soils under investigation have CEC values ranging from medium to very high (Hazelton and Murphy, 2016), which suggests sufficient buffering and nutrient retention. But across soil depths, there was no consistent trend in the cation exchange capacity beneath the two pedons. A comparison of the Jara Gelalicha and Aruma surface horizons revealed that the latter has a higher cation exchange capacity (CEC). This difference in CEC can be attributed to the presence of organic carbon in the soil, as well as the relatively high clay concentration. The soil in the research area, with its high CEC, has an excellent capacity to buffer and retain nutrients. However, CEC did not show a consistent pattern at various soil depths. According to Landon (1991), soils with CEC values less than 5, 5–15, 15–25, 25–40, and greater than 40 cmol(+)/kg are classified as very low, low, medium, high, and very high, respectively. The CEC values of the soils studied were typically in the low to high range (Hazelton and Murphy, 2016), indicating strong nutrient retention and buffering capability. However, the cation exchange capacity under the two pedons showed an unsystematic tendency along soil depths.

In decreasing sequence,  $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$  were the predominant cations filling the exchange sites. The calcium (Ca) concentration decreased with increasing soil depth in the Jara Gelalicha and Aruma locations (Table 2.5). In the surface layer of Jara Gelalicha and Aruma, exchangeable calcium values were 8.23 cmol (+)/kg and 10.95 cmol (+)/kg, respectively (Table 2.5). In the subsurface horizons, exchangeable Ca ranged from 11.78 cmol (+) /kg to 14.91 cmol (+) /kg and 10.95 to 4.32 cmol (+) /kg (Table 2.5). In Jara Gelalicha, exchangeable Ca increased with depth, while at Aruma, Ca had no systematic trend with depth. The pH value of 5 to 9 was the most ideal pH range for the obtainability of  $\text{Ca}^{2+}$ , and the highest concentration of  $\text{Ca}^{2+}$  showed the status of weathering (Hazelton and Murphy, 2016; Dinssa and Elias, 2021; Mathwose and Mesfin, 2024). The Ca: Mg ratios of the surface layers in the Jara Gelalicha and Aruma areas were 3.9:1 and 3.8:1, respectively, falling within the (1-4:1) low Ca value range for most crop production. According to Hazelton and Murphy (2016), a Ca: Mg ratio less than 4:1 resulted in low Ca availability, indicating a scarcity of Ca uptake due to an excess of Mg or washing away basic cations due to the high amount of rainfall. According to Hazelton and Murphy (2016), the Ca: Mg ratio in both pedons of the surface soils was less than 4:1, which meant that there was a limited supply of calcium. This might be because excessive magnesium prevents the body from absorbing calcium, or excessive rainfall can wash out the basic cations (Sebnie et al., 2021; Mathwos and Mesfin, 2024). Calcium and magnesium were the main basic

cations found in the soils; these findings are consistent with previous studies by Sharu et al. (2013), Paramasivan and Jawahar (2014), and Kebede et al. (2017). In Jara Gelalicha's profile, there was an increase in trends in the value of exchangeable calcium related to soil depth. This could be a result of parent materials containing calcium being distributed regularly throughout the soil profiles (Sebnie et al., 2021). The Aruma soils had lower values of exchangeable bases, while the Jara Gelalicha soils showed higher values (Table 2.5). The Aruma area generally showed an irregular trend in cation exchange capacity with profile depth, which may have resulted from a strong correlation between OC and cation exchange capacity. This result is consistent with Kedir (2015), who reported that variations in organic matter account for variations in cation exchange capacity, i.e., an increase in organic matter led to an increase in CEC.

Exchangeable potassium (K) and sodium (Na) in Jara Gelalicha ranged from 1.17 to 2.77 and 0.85 to 1.76 cmol(+)/kg, respectively, while the contents of exchangeable calcium and magnesium changed from the surface to subsurface from 11.78 to 14.91 cmol(+)/kg and 3.17 to 11.84 cmol(+)/kg, respectively (Table 2.5). This finding aligns with Kedir's (2015) research, which indicated that variations in organic matter contribute to changes in cation exchange capacity, meaning an increase in organic matter leads to a higher CEC. According to Hazelton and Murphy (2007), the exchangeable calcium in the Jara Gelalicha soils was rated as high, while the values in the Jara Gelalicha and Aruma soils ranged from high to medium to high (Table 2.5). Exchangeable calcium was the most dominant cation at the exchange site in Jara Gelalicha, followed by magnesium (Mg), potassium (K), and sodium (Na) across the pedon horizon (Table 2.5), demonstrating a suitable basic cation distribution according to FAO (2014).

Table 2.5. Exchangeable bases, cation exchange capacity, total exchangeable base, Ca: Mg ratio and percent base saturation of soil profiles of the study areas.

Depth(cm)	Horizon	Exchangeable cations (cmol(+)/kg)				Sum (cmol(+)/kg)	CEC (cmol(+)/kg)	Ca:Mg	PBS(%)
		Ca	Mg	K	Na				
Jara Gelalicha									
0-18	Ap	8.23	3.17	1.17	0.85	13.42	15.83	2.60	84.78
18-40	AB	11.78	3.66	1.29	0.89	15.15	15.64	3.49	72.00
40-80	Bw	12.34	4.38	2.61	1.34	12.07	13.07	3.05	75.00
80-110	BC1	13.76	4.50	2.67	1.56	22.49	37.02	3.06	61.00
110-160	BC2	14.83	5.40	2.72	1.76	22.71	39.24	2.75	58.00
160-200 <sup>+</sup>	C	14.91	5.84	2.77	1.30	22.82	39.32	2.55	58.00
Aruma									
0-20	Ap	10.95	2.84	0.55	0.20	13.24	24.08	3.86	55.00
20-70	AB	13.05	2.56	0.25	0.24	16.10	26.21	5.09	61.08
70-115	B1	12.27	2.71	0.27	0.29	15.54	24.14	4.53	64.34
115-140	B2	11.07	2.04	0.26	0.43	13.80	26.80	5.42	51.49
140-164	2BC	10.57	1.94	0.26	0.43	13.20	27.14	5.45	49.00
164-200 <sup>+</sup>	2C	4.32	2.08	0.19	0.58	7.17	23.12	2.07	44.00

CEC= cation exchange capacity, PBS= percent of base saturation

### 2.3.3.4. Extractable micronutrient contents of soils

The available micronutrient contents all showed irregular trends with increasing soil depth in both areas, except for Mn, which showed a decreasing trend with increasing depth in Aruma (Table 2.6). In the both area pedons, the extracted micronutrient concentrations were Mn>Fe>Zn>Cu. In the Jara Gelalicha, available iron (Fe) ranged from 1.14 to 3.06 mg kg<sup>-1</sup> in subsurface horizons and 2.27 mg kg<sup>-1</sup> in the surface soil horizon. In the Aruma, Fe available in the surface horizons was 3.78 mg kg<sup>-1</sup>, while available Fe in the irregular trend in Fe concentration as the depth increased. In the Jara Gelalicha area, available manganese (Mn) ranged from 2.87 to 7.68 mg kg<sup>-1</sup> in subsurface horizons and 6.02 mg kg<sup>-1</sup> in the surface soil horizon. In the Aruma area, available Mn ranged from 1.397 to 24.51 mg kg<sup>-1</sup> in subsurface horizons and 34.11 mg kg<sup>-1</sup> in surface horizons. Many factors influence soil micronutrient content, with the most important being soil organic matter, moisture, soil aeration, soil pH, and clay content (Ashenafi et al., 2010). According to Jones (2003), the available Zn concentrations extracted from the surface horizons of the Jara Gelalicha and Aruma locations were considered moderate (marginal) and high (sufficient), respectively. Zinc content below 0.5 mg Zn kg<sup>-1</sup> was considered insufficient by the authors, possibly due to the high pH that leads to zinc sorption caused by the presence of high CaCO<sub>3</sub> (Najafi-Ghiri et al., 2013).

Table 2.6. Extractable micronutrient contents of Jara Gelalicha and Aruma areas of soil

Depth(cm)	Horizon	Fe	Mn mg kg <sup>-1</sup>	Zn	Cu
Jara Gelalicha					
0-18	Ap	2.27	6.02	0.58	0.27
18-40	AB	3.06	4.76	0.63	0.29
40-80	Bw	2.87	7.55	0.78	0.16
80-110	BC1	1.48	6.37	0.40	0.23
110-160	BC2	2.56	7.68	0.53	0.30
160-200 <sup>+</sup>	C	1.14	2.87	0.32	0.27
Aruma					
0-20	Ap	3.78	34.11	0.65	0.33

20-70	AB	6.1	24.51	0.72	0.36
70-115	B1	8.4	14.48	0.62	0.28
115-140	B2	4.06	5.805	0.60	0.25
140-164	2BC	4.52	4.46	0.37	0.27
164-200 <sup>+</sup>	2C	10.28	1.397	0.36	0.43

#### 2.3.4. Pearson correlation coefficients

The research revealed that certain soil physicochemical properties had substantial associations with one another, while others did not have relevant relationships in both sites (Table 2.7). In the Jara Gelalicha and Aruma areas, among the positively correlated parameters, organic carbon (OC) was significantly correlated with clay ( $r = 0.738^*$ ,  $0.831^{**}$ ), total nitrogen (TN) ( $r = 0.892^{***}$ ,  $0.981^{***}$ ), available phosphorus (Ava.P) ( $r = 0.984^{***}$ ,  $0.973^{***}$ ), calcium with CEC, and Mg with CEC and pH, respectively (Table 2.7). These positive associations indicate that organic carbon improves the soil's ability to retain nutrients and overall soil fertility. For example, TN and Ava.P, both important for plant growth, have strong positive associations with OC, showing that higher organic content improves nutrient availability in the soil. On the other hand, some parameters showed negative correlations. In Jara Gelalicha and Aruma sites, clay showed a significant negative correlation with bulk density (BD) ( $r = -0.110$ ,  $-0.421$ ), respectively (Table 2.7). Bulk density indirectly measures soil porosity, indicating that higher clay content tends to reduce the total pore space in the soil, possibly due to the smaller size of clay particles and their tendency to compact. Similarly, Ca showed a negative correlation with sand ( $r = -0.040$ ,  $-0.023$ ), suggesting that an increase in calcium might decrease sand. These relationships highlight how certain soil properties can inhibit or promote others, underlining the complex interdependence of soil chemistry and structure (Table 2.7). A similar result was reported by Mengiste et al. (2025). Therefore, this indicates that as organic carbon increases, the bulk density of soil decreases.

Table 2.7. Correlation matrix for linear relationships between soil physicochemical properties of the study areas

Para	Aruma area												
	Sand	Clay	BD	EC	pH	CEC	OC	TN	Ava.P	Ca	Mg	K	Na
Sand	1	-	0.426	0.019	0.252	0.051	-0.562	-0.46	-0.512	-0.023	0.303	0.061	0.211
Clay	-	1	-0.421	-0.641*	-0.621*	-0.270	0.831*	0.886*	0.730	-0.271	-0.366	-0.439	-
BD	0.290	-0.110	1	-0.523*	-0.454	-0.562*	-0.247	-0.014	-0.325	-0.65**	-0.435	-	-
EC	0.291	-	-	1	0.811**	0.873**	-0.440	-	-0.124	0.814**	0.843	0.813*	0.832
pH	0.276	-	-0.352	0.924**	1	0.736**	-0.320	-	-	0.822**	0.876	0.921*	0.943
CEC	0.076	-0.324	-	0.920**	0.910**	1	0.745*	-	-0.298	0.880**	0.954	0.841*	0.802
OC	-0.422	0.738*	-0.389	-0.362	-0.610*	-0.317	1	0.981*	0.973	-0.021	-0.468	-	-
TN	-0.60*	0.854*	-0.129	-0.680*	-	-0.598*	0.892*	1	0.942	-0.468	-	-	-
Av.p	-0.414	0.709*	-0.417	-0.317	-0.579*	-0.298	0.984*	0.865*	1	-0.144	-0.351	-0.423	-
Ca	-0.040	-0.296	-	0.914**	0.847**	0.880**	-0.188	-0.468	-0.148	1	0.893	0.970*	0.883
Mg	0.211	-0.446	-	0.965**	0.948**	0.954**	-0.406	-	-0.373	0.905**	1	0.931*	0.864

K	0.092	-0.479	-	0.923**	0.891**	0.841**	-0.374	-	-0.337	0.970**	0.904	1	0.924
			0.683	*				0.612*			***		***
Na	0.256	-	-	0.902**	0.920**	0.802**	-	-	-	0.883**	0.864	0.953*	1
		0.625*	0.513	*	*		0.548*	0.760*	0.515	*	****	***	
			*						*				

Jara Gelalicha area

Numbers are Pearson correlation coefficients (r). Number of observation (n) = 12; significant at  $p \leq 0.05$ ,  $**p \leq 0.01$ , and  $***p \leq 0.001$ . BD = bulk density; EC= electrical conductivity; OC = organic carbon; TN= total nitrogen; Av. P = available phosphorus; CEC = cation exchange capacity.

### 2.3.5. Classification of soil of the study areas

#### 2.3.5.1. Soil Classification based on WRB Legend

Using the IUSS Working Group WRB (2022) classification, the soils in the study areas were classified based on morphological and physicochemical characteristics. The pedon opened in Jara Gelalicha on level land, had a silt/clay ratio of  $>1$ , and showed evidence of pedogenic alteration without significant accumulation of illuviated materials like clay or organic matter, which is a typical feature of a cambic subsurface diagnostic horizon. These characteristics met the criteria for Cambisols as a reference soil group. According to Dinnsa and Elias (2021) and Ahukaemere et al. (2017), soils with a silt/clay ratio of less than 0.15 are considered weathered, while Cambisols with a Si/C ratio of more than 0.15 are younger and have a higher potential for weathering. The silt/clay ratio is significant in determining soil weathering because soils with a higher silt/clay ratio are less weathered and contain more clay minerals like smectite, resulting in high water retention and plant-available water capacity (Ivory et al., 2014). The pedon had a high ( $>50\%$ ) percent base saturation that qualifies for the Eutric principal qualifier. The textural classes of the horizons of the pedon were sandy loam, sandy clay loam and loam which qualify the requirement of Loamic supplementary qualifier. Thus, the pedon was classified as Eutric Cambisols (Loamic).

The surface horizon of the pedon in Aruma area had high organic carbon (3.57%); a Munsell soil color value of  $\leq 3$  moist and  $\leq 5$  dry; and a chroma of  $\leq 3$  moist. As a result, the pedon had a mollic surface diagnostic horizon. The surface horizon of the pedon had low bulk density ( $0.87 \text{ g/cm}^3$ ) and high (67.29%) porosity. According to Temesgen et al. (1999), the Aruma area contains volcano-lacustrine deposits with basaltic lava. Therefore, the soil of the study area was classified as Andosols, volcanic ash soils. The pedon had a high ( $>50\%$ ) percent base saturation that qualifies for the Eutric principal qualifier. The textural classes of the horizons of the pedon were sandy clay loam and clay loam, which qualify the requirement of Loamic supplementary qualifier. Therefore, the pedon was classified as Eutric Andosols (Loamic) (Table 2.8).

Table 2.8. Diagnostic horizons and soil units at two locations according to WRB (2022).

Location	Pedon	Diagnostic horizons		Soil Unit	Reference Soil Group
		Surface	Subsurface		
Jara Gelalicha	1	-	Cambic	Eutric Cambisols(Loamic)	Cambisols
Aruma	1	Mollic	Cambic	Eutric Andosols (Loamic)	Andosols

## 2.4. Conclusions

The soils of the two sites had different morphological, physical, and chemical properties, leading to different reactions to management practices, abilities to provide ecosystem services naturally, resilience to disturbance, and susceptibility to deterioration. The surface horizon textural class of the pedon in Jara Gelalicha was sandy loam, whereas the textural class of the pedon at Aruma was sandy clay loam. The surface horizon OC of Aruma (3.57%) was higher than Jara Gelalicha (2.39%). Likewise, the total nitrogen content of the surface horizon of Aruma (0.32%) was greater than Jara Gelalicha (0.23%). Similarly, the CEC value of the surface horizon at Aruma (24.08 cmol(+)/kg) was greater than Jara Gelalicha (15.83 cmol(+)/kg). The Jara Gelalicha pedon was classified as Eutric Cambisols (Loamic), whereas the Aruma pedon was classified as Eutric Andosols (Loamic). Therefore, the soil fertility management and land use decisions should consider such soil variability.

## 2.5. References

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## CHAPTER THREE

### 3. INFLUENCE OF AZOLLA DRY BIOMASS AND INORGANIC NITROGEN FERTILIZER ON SOIL CHEMICAL PROPERTIES, YIELD AND YIELD COMPONENTS OF TOMATO UNDER GREENHOUSE CONDITIONS

#### Abstract

*Low soil fertility and inadequate fertilizer application can limit tomato production. Therefore, this experiment was conducted at a greenhouse in School of Plant and Horticultural Sciences, Hawassa University, College of Agriculture. The aim of this study was to investigate the impacts of Azolla dry biomass and inorganic N-fertilizer applications on soil chemical properties soil samples were collected at a depth of 0-20 cm from the Hawassa Zuria and Wondo Genet locations as well as to determine the optimum combination of Azolla dry biomass and N-fertilizer for maximizing tomato yield. The study used a factorial combination of four rates of Azolla dry biomass (0, 25, 50, and 75 g pot<sup>-1</sup>) and four levels of inorganic N (0, 0.23, 0.46, and 0.69 g pot<sup>-1</sup>) in a completely randomized design with three replications. Data were collected on selected chemical soil properties, tomato yield and yield component parameters. Analysis of variance was conducted using the General Linear Model (GLM) to analyze the data. The means were then separated using the LSD test at a significance level of 5%. The results showed that the interaction effect of Azolla and inorganic N had a significant influence on yield and yield component in both collected soils. The results showed that Azolla dry biomass and nitrogen fertilizer had a significant impact on tomato growth and yield. Compared to the control group, the combined application of Azolla dry biomass (75 g pot<sup>-1</sup>) and N (0.69 g N pot<sup>-1</sup>) resulted in the highest marketable fruit yields of 1088.43 g/plant in soil collected from Hawassa Zuria and 833.73 g/plant in soil collected from Wondo Genet. Therefore, applying 75 g pot<sup>-1</sup> (15 t ha<sup>-1</sup>) of Azolla along with 0.69 g pot<sup>-1</sup> (138 kg ha<sup>-1</sup>) of N fertilizers suggests that this fertilizer combination leads to improved tomato production and soil fertility. Specifically, the soil collected from Hawassa Zuria and Wondo Genet sites showed that the integrated application of Azolla dry biomass (75 g pot<sup>-1</sup>) and inorganic nitrogen (0.69 g N pot<sup>-1</sup>) resulted in maximum soil total nitrogen levels (0.32% and 0.45%), organic carbon content (4.38% and 5.04%), available phosphorus (19.68 mg kg<sup>-1</sup> and 35.40 mg kg<sup>-1</sup>), and cation exchange capacity (29.50 cmol(+) kg<sup>-1</sup> and 37.37 cmol(+) kg<sup>-1</sup>) compared to the control group. Field research is necessary to confirm the greenhouse results and provide appropriate recommendations.*

**Keywords:** Macronutrient, organic nitrogen, plant growth, soil fertility, soil nutrient

#### 3.1. Introduction

Soil fertility decline is one of the most significant constraints to increased food production in Ethiopia (Aleminew and Alemayehu, 2020). It has been a challenging and limiting factor for food

security in the country (Ocho et al., 2017). The primary causes of soil fertility decline include the loss of organic matter (OM), depletion of macro and micronutrients, soil acidity, topsoil erosion, and deterioration of physical soil properties (Musa et al., 2024). Anthropogenic factors such as inappropriate land use systems, nutrient mining, and inadequate nutrient supply have worsened the situation (Hossain et al., 2020). To increase soil fertility in the short term, nutrients need to be added to the soil, often through the application of inorganic fertilizers (Bhatt et al., 2019). However, chemical fertilizers can be expensive to purchase, posing a problem for most small-scale farmers (Alemineu and Alemayehu, 2020). Inorganic fertilizers are crucial sources of major elements in crop production (Bhatt et al., 2019). Continuous use of inorganic fertilizers has led to imbalances in soil physicochemical properties, and unsustainable crop production (Jeyathilake et al., 2006). To ensure soil productivity, plants need an adequate and balanced supply of nutrients, which can be achieved through integrated nutrient management that utilizes both natural and man-made sources of plant nutrients (Panta and Parajulee, 2021). Combining organic and inorganic fertilizers results in greater benefits than using either input alone, as they have positive interactions on soil biological, chemical, and physical properties (Bekunda et al., 2010).

Tomato (*Solanum Lycopersicon* L.) is one of the most widely cultivated vegetable crops globally and holds major importance in Ethiopia (Sigaye and Mekuria, 2022). It is the third most important vegetable after potato and sweet potato, and ranks first as a processing crop (FAO, 2010). China leads global production with 58.968 million tons annually (FAOSTAT, 2017). Despite this global significance, Ethiopia's average tomato yield is only 8 t ha<sup>-1</sup>, far below the world average of 34 t ha<sup>-1</sup> (FAOSTAT, 2012), and cultivated area has declined from 6,298.63 ha in 2016/17 to 5,235.19 ha in 2017/18 (Beyene and Mulu, 2019). Poor fertilizer utilization further disrupts soil nutrient balance and reduces productivity across Africa, including Ethiopia (Tesfay et al., 2018). Tomato production on low-fertility soils depends on increasing soil organic matter to improve soil properties and nutrient availability. However, inadequate fertilizer application, pest and disease pressure, moisture stress, and extreme temperatures severely limit productivity (Etissa et al., 2013). Enhancing soil organic matter can reduce reliance on mineral fertilizers and support sustainable production (Fawzy et al., 2016; Zeleke et al., 2024). Overuse of inorganic fertilizers contributes to environmental degradation, soil fertility decline, reduced yields, and erosion, while their high cost and limited availability delay application for many farmers. Conversely, organic fertilizers are more accessible (Kashem et al., 2015). Overall, the main

constraints to tomato production in Ethiopia include insufficient fertilizer use, pests and diseases, high postharvest losses, limited adoption of improved technologies, and weak market systems (Getahi, 2020).

Nitrogen is an essential nutrient for plant growth and increased agricultural production. With the global population projected to reach 9.7 billion by 2050, demand for nitrogen fertilizers is predicted to rise (United Nations, 2023). However, the widespread use of inorganic fertilizers has raised a number of environmental concerns, including soil deterioration, increased salinity, and nutrient runoff into aquatic bodies (Tyagi et al., 2022). The use of Azolla as an organic fertilizer has been on the rise recently due to its high nitrogen content (Zbytniewski et al., 2005). Azolla provides an environmentally friendly option by boosting nitrogen use efficiency, soil health, and microbial diversity (Fitriatin et al., 2021). These advantages lower the need for inorganic fertilizers and contribute to long-term soil fertility (Yang et al., 2022). Moreover, Azolla breaks down efficiently and rapidly, increasing the amount of nitrogen available for crop growth. According to Skorupka and Nosalewicz (2021), it also has a high protein content, act as an herbicide, and can reduce the volatilization of nitrogen fertilizer. However, there is limited information available on the combined effect of Azolla dry biomass and inorganic N nitrogen fertilizers combination on tomato yield, yield components, and their contribution to improving soil chemical properties. Therefore, the aim of the current study is to determine the effects of Azolla dry biomass and inorganic N combinations on soil chemical properties and tomato yield as well as to determine the optimal combinations of treatments for maximizing tomato yield.

## **3.2. Materials and Methods**

### **3.2.1. Description of the study areas**

The pot experiment was conducted at Hawassa University, College of Agriculture, School of Plant and Horticultural Sciences, in a greenhouse. The soil samples were collected at a depth of 0-20 cm from the Hawassa Zuria and Wondo Genet districts of the Sidama Region, Southern. The relative humidity of the greenhouse is 75%. The temperature in the greenhouse ranged from 21-23°C. The first soil sample was collected at Jara Galalicha Kebele, on a farmer's farm in Hawassa Zuria district. The second soil sample was collected at Aruma Kebele, on a private farm in Wondo Genet district. An improved tomato variety named Gelilema (*Lycopersicon esculentum* L.) was used as a test crop.

### 3.2.2. Experimental design, treatments, and procedures

The experiment was conducted under greenhouse conditions in a pot. For the greenhouse pot experiment, ninety-six (96) pots of 45 cm in diameter and 50 cm deep were used, and each pot was filled with 10 kg of 2 mm sieved soil. Both urea (nitrogen) and triple superphosphate (TSP) fertilizers were obtained from Hawassa Agricultural Research Center. Azolla as an organic source of N fertilizer was obtained from the School of Animal and Range Science, College of Agriculture, Hawassa University. Treatment-wise, Azolla as an organic N source was incorporated into the prepared soil containing experimental pots 15 days before transplanting.

A combination of four rates of Azolla dry biomass (0, 25, 50, and 75 g pot<sup>-1</sup>) as an organic nitrogen source and four levels of inorganic N fertilizer (0, 0.23, 0.46, and 0.69 g N pot<sup>-1</sup>) was evaluated. Urea is used as a source of inorganic N, while TSP is used as a source of phosphorous fertilizers. The treatments were set up in a factorial combination within a completely randomized design (CRD) with three replications. There were a total of 48 experimental units for each collected soil location and 96 experimental units for two locations. Each treatment consisted of one pot, with two plants per pot, totaling 192 plants per location, and the spacing between pots in a row was 50 cm. Urea, with nitrogen (N) content of 46%, served as the inorganic nitrogen source. In the nursery, seeds of tomato *Gelilema* were sown on a flatbed for one month before transplanting to the pots. Thirty-five (35) day-old and uniform tomato seedlings with 2-3 leaves were transplanted into each pot. It is recommended to transplant either early in the morning or late in the evening.

Triple superphosphate (TSP) was used as the source of phosphorus (P), and the entire rate was applied via band application for all pots after transplanting. While urea was applied in two equal splits, half dose at transplanting and half at 35 days after transplanting, the amount based on the treatment. All of the Azolla dry biomass was broadcasted 15 days before transplanting except for the control, which was incorporated into the soil at a plow depth of 15 cm. 0.23 mg P<sub>2</sub>O<sub>5</sub> pot<sup>-1</sup> soil from TSP was added to each pot at transplanting times for all plots. Water was regularly applied to each pot up to field capacity. Following standard media preparation practices, the pots were filled with 10 kg of 2 mm sieved soil 15 days before planting. Weeding was done as needed to keep the pots free of weeds, and the plants were allowed to grow. Tomato pest and disease management involves cultural practices, biological agents, and need-based chemical sprays to

control major pests such as whiteflies, and aphids, as well as diseases like early and late blight and wilt disease.

### **3.2.3. Soil sampling and soil analysis**

Before setting up the treatment, soil samples were collected from the surface layer (0-20 cm deep) in the Hawassa Zuria and Wondo Genet districts. Different subsamples or individual auger samples were randomly taken from various positions in the experimental field in a zigzag pattern at each location. These samples were then mixed to create one composite sample at each location. Before planting in the pot, composite soil samples were analyzed from soil collected both locations. Soil samples were collected from each treatment of the experimental pot after harvest at the end of the growing season. An auger was used to collect soil samples for all soil analysis parameters. Particle size distribution (soil texture) was determined using the modified Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was measured potentiometrically with a combined glass electrode pH meter in a 1:2.5 soil-to-water suspension (Van Reeuwijk, 1992). Electrical conductivity (EC) was measured using a conductivity meter in a soil–water extract (Okalebo et al., 2002). Cation exchange capacity (CEC) was determined by saturating the soil with 1 M  $\text{NH}_4\text{OAc}$  (pH 7), washing with ethanol, and then displacing adsorbed ammonium with sodium ions (Chapman et al., 1961). The released ammonium in the distillate was back-titrated with dilute acid, and the CEC was expressed as the amount of ammonium exchanged. Organic carbon (OC) content was measured by the wet digestion chromic acid method described by Walkley and Black (1934). Total nitrogen was determined using the Kjeldahl digestion procedure as outlined by Jackson (1958). Available phosphorus (Ava.P) was extracted following the standard Olsen method (Olsen et al., 1954).

### **3.2.4. Data collection**

All crop greenhouse data was collected. Vegetative growth and yield components of tomatoes were collected following proper procedures. Vegetative growth parameters measured included plant height (cm), leaf area ( $\text{cm}^2$ ), and the number of branches. Yield and yield components measured included the number of fruits per cluster, the number of flowers, the number of fruits per plant, fruit weight per plant (g), and marketable fruit yield ( $\text{t ha}^{-1}$ ) and fruit yield ( $\text{t ha}^{-1}$ ). Plant height (cm) was recorded after transplanting (60 DAT). The number of fruits was counted and weighed after harvesting.

### **3.2.5. Data analysis**

The soil chemical properties and agronomic data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) in SAS version 9.4 software. Azolla dry biomass and inorganic nitrogen fertilizers are considered fixed factors, while the location of collected soil is considered random factor. Mean separation was conducted based on the least significant difference (LSD) at a significance level of 5% (SAS Institute, 2016).

### 3.3. Results and Discussion

#### 3.3. 1. Effects of Azolla dry biomass and inorganic N fertilizers on the chemical properties of the soil after harvest

In both soils types, organic carbon, total nitrogen, available phosphorus, and CEC were significantly affected by the combined application of Azolla dry biomass and inorganic nitrogen rates after harvest ( $P < 0.01$ ). Soil collected from Hawassa Zuria showed that the interaction effect of Azolla dry biomass and inorganic nitrogen fertilizer rates had a significant impact on soil pH ( $P < 0.05$ ). As the soil pH decreases due to Azolla biomass decomposition, it adds organic matter to the soil. The breakdown of organic materials can produce organic acids, including humic and fulvic acids, as byproducts of the decomposition process (Chang et al., 2023). However, the integrated application of Azolla dry biomass and inorganic nitrogen rates had no significant effect on soil electrical conductivity (EC) compared to the control in both locations. Additionally, the combined application of Azolla dry biomass and nitrogen rates had no significant effect on soil pH in soil collected from the Wondo Genet location (Table 3.1).

The combined application of Azolla dry biomass and inorganic nitrogen fertilizers improves soil chemical properties (Table 3.1). A similar result was reported by Phillips et al. (2022), indicating that using both organic and inorganic nitrogen fertilizers together can enhance the soil's organic carbon, total nitrogen (N), available phosphorus, and cation exchange capacity. Organic fertilizers improve the quality and availability of nutrients in the soil by adding organic matter. In soil collected from Hawassa Zuria and Wondo Genet locations, the highest values of CEC were recorded from the combined application of  $75 \text{ g pot}^{-1}$  Azolla dry biomass with  $0.69 \text{ g N pot}^{-1}$ , with values of  $29.50 \text{ cmol(+) /kg}$  and  $37.37 \text{ cmol(+) /kg}$ , respectively, compared to control treatments (Table 3.1). The study by Korsá et al. (2024), which found that Azolla-treated paddy soils improved CEC by 28–35%, mostly as a result of enhanced humic acid generation, is in line with the observed increase in CEC after Azolla integration. Furthermore, Akhtar et al. (2021) showed that the breakdown of Azolla releases organic acids that promote CEC; however, their research did not measure the precise amount of Azolla that improves CEC.

Soil collected from Hawassa Zuria and Wondo Genet locations showed that the combined application of  $75 \text{ g pot}^{-1}$  Azolla dry biomass and  $0.69 \text{ g N pot}^{-1}$  resulted in maximum soil organic carbon (OC) contents (4.38% and 5.04%, respectively), compared to the control group (Table

3.1). The findings suggest that the combined application of Azolla dry biomass at  $75 \text{ g pot}^{-1}$  with  $0.69 \text{ g N pot}^{-1}$  increased the organic carbon by 52.92% in soil collected from Hawassa Zuria and by 36.31% in Wondo Genet compared to the control group ( $p < 0.05$ ). A similar result was reported by Adnyana et al. (2025), showing that Azolla incorporation led to a 29% increase in soil OC compared to the control group. The increase in soil OC from the applied treatments may be attributed to the improvement of soil organic matter. These results are consistent with the findings of Marzouk et al. (2024), who reported an increase in soil OC content after harvest with the combined application of Azolla and inorganic NP in rice compared to the control. Furthermore, Mayly and Hidayat (2024) reported that the application of Azolla microphylla in rice increased soil physiochemical characteristics and promoted rice growth.

Table 3. 1. Interaction effects of Azolla dry biomass and inorganic N fertilizers on soil chemical properties of soil after harvest in the study areas

Treatments		Soil from Hawassa Zuria						Soil from Wondo Genet					
		Soil pH	EC(dS/m)	Total N (%)	OC (%)	Ava. P(mg kg <sup>-1</sup> )	CEC /kg	Soil pH	EC(dS/m)	Total N (%)	OC (%)	Ava. P(mg kg <sup>-1</sup> )	CEC /kg
0	0	8.63 <sup>a</sup>	3.04	0.16 <sup>m</sup>	2.15 <sup>h</sup>	9.72 <sup>k</sup>	21.09 <sup>gh</sup>	6.99	1.74	0.30 <sup>g</sup>	3.21 <sup>j</sup>	28.85 <sup>kl</sup>	23.49 <sup>f</sup>
	0.23	8.54 <sup>abc</sup>	3.09	0.19 <sup>jk</sup>	2.53 <sup>g</sup>	11.31 <sup>ghi</sup>	20.54 <sup>h</sup>	6.75	1.78	0.36 <sup>f</sup>	3.71 <sup>i</sup>	30.64 <sup>j</sup>	25.41 <sup>f</sup>
	0.46	8.48 <sup>cd</sup>	3.17	0.20 <sup>ij</sup>	3.56 <sup>bc</sup>	11.94 <sup>fgh</sup>	21.62 <sup>fgh</sup>	6.92	2.04	0.37 <sup>de</sup> <sub>f</sub>	4.18 <sup>gh</sup>	31.25 <sup>hi</sup>	29.14 <sup>de</sup>
	0.69	8.60 <sup>ab</sup>	3.04	0.23 <sup>gh</sup>	3.09 <sup>ef</sup>	9.73 <sup>k</sup>	22.79 <sup>ef</sup>	6.87	1.83	0.40 <sup>bc</sup> <sub>d</sub>	4.26 <sup>fg</sup>	31.00 <sup>ij</sup>	31.06 <sup>bcd</sup>
25	0	8.61 <sup>ab</sup>	2.92	0.18 <sup>kl</sup>	3.57 <sup>bc</sup>	11.32 <sup>ghi</sup>	24.38 <sup>cd</sup>	6.64	2.02	0.41 <sup>bc</sup>	4.25 <sup>fg</sup>	30.63 <sup>j</sup>	30.77 <sup>cde</sup>
	0.23	8.50 <sup>bc</sup>	2.80	0.23 <sup>gh</sup>	3.03 <sup>ef</sup>	9.76 <sup>k</sup>	20.51 <sup>h</sup>	6.85	1.72	0.42 <sup>b</sup>	4.16 <sup>gh</sup>	28.89 <sup>k</sup>	32.36 <sup>abc</sup>
	0.46	8.44 <sup>cde</sup>	2.96	0.26 <sup>ef</sup>	2.65 <sup>fg</sup>	13.15 <sup>cde</sup>	23.90 <sup>de</sup>	6.53	1.90	0.37 <sup>ef</sup>	4.33 <sup>def</sup>	31.26 <sup>hi</sup>	32.73 <sup>abc</sup>
	0.69	8.37 <sup>def</sup>	3.07	0.27 <sup>de</sup>	3.38 <sup>de</sup>	12.58 <sup>ef</sup>	24.44 <sup>c</sup>	6.69	1.72	0.41 <sup>bc</sup>	4.56 <sup>cd</sup>	32.79 <sup>f</sup>	32.75 <sup>abc</sup>
50	0	8.29 <sup>f</sup>	2.88	0.27 <sup>de</sup>	3.36 <sup>cd</sup>	11.33 <sup>ghi</sup>	24.46 <sup>bc</sup>	6.58	2.25	0.37 <sup>ef</sup>	4.52 <sup>d</sup> <sub>h</sub>	32.24 <sup>fg</sup> <sub>h</sub>	33.08 <sup>ab</sup>
	0.23	8.35 <sup>ef</sup>	2.98	0.29 <sup>c</sup>	3.44 <sup>ed</sup>	16.21 <sup>c</sup>	22.55 <sup>efg</sup>	6.62	1.91	0.37 <sup>ef</sup>	4.43 <sup>de</sup>	32.42 <sup>fg</sup>	32.85 <sup>abc</sup>
	0.46	8.37 <sup>def</sup>	2.83	0.31 <sup>ab</sup>	3.62 <sup>cd</sup>	17.13 <sup>bc</sup>	25.76 <sup>bc</sup>	6.83	2.05	0.39 <sup>cd</sup>	4.71 <sup>c</sup>	32.77 <sup>f</sup>	32.36 <sup>abc</sup>

e													
	0.69	8.18 <sup>g</sup>	2.67	0.28 <sup>cd</sup>	3.46 <sup>cd</sup>	18.14 <sup>b</sup>	23.46 <sup>de</sup>	6.62	1.85	0.41 <sup>bc</sup>	4.34 <sup>def</sup>	33.76 <sup>e</sup>	29.53 <sup>de</sup>
75	0	8.07 <sup>h</sup>	2.90	0.22 <sup>hi</sup>	3.53 <sup>cd</sup>	12.38 <sup>ef</sup>	24.64 <sup>cd</sup>	6.71	1.82	0.40 <sup>bc</sup>	4.48 <sup>d</sup>	34.91 <sup>c</sup>	29.14 <sup>de</sup>
	0.23	7.54 <sup>i</sup>	2.82	0.32 <sup>a</sup>	3.89 <sup>bc</sup>	14.07 <sup>d</sup>	25.87 <sup>bc</sup>	6.87	1.99	0.36 <sup>f</sup>	4.69 <sup>c</sup>	34.14 <sup>d</sup>	28.57 <sup>de</sup>
	0.46	7.43 <sup>j</sup>	2.67	0.31 <sup>ab</sup>	4.02 <sup>b</sup>	16.31 <sup>c</sup>	26.53 <sup>b</sup>	6.68	2.04	0.42 <sup>b</sup>	5.02 <sup>ab</sup>	35.88 <sup>b</sup>	32.57 <sup>abc</sup>
	0.69	7.31 <sup>kl</sup>	2.50	0.32 <sup>a</sup>	4.38 <sup>a</sup>	19.68 <sup>a</sup>	29.50 <sup>a</sup>	6.60	1.92	0.45 <sup>a</sup>	5.04 <sup>a</sup>	37.37 <sup>a</sup>	33.40 <sup>a</sup>
<b>LSD(0.05)</b>		<b>0.10</b>	<b>Ns</b>	<b>0.012</b>	<b>0.37</b>	<b>1.15</b>	<b>1.56</b>	<b>ns</b>	<b>ns</b>	<b>0.026</b>	<b>0.16</b>	<b>0.74</b>	<b>1.12</b>
<b>CV (%)</b>		<b>5.63</b>	<b>7.53</b>	<b>8.32</b>	<b>13.86</b>	<b>5.59</b>	<b>3.90</b>	<b>2.11</b>	<b>9.04</b>	<b>4.03</b>	<b>3.17</b>	<b>2.76</b>	<b>4.50</b>

EC: electrical conductivity; OC: organic carbon; Ava. P: available phosphorus; CEC: cation exchange capacity. Means in the same column followed by the same letter(s) are not significantly different ( $P \leq 0.05$ ).

### **3.3.2. Effects of Azolla dry biomass and inorganic N fertilizers on tomato growth and yield components**

#### **3.3.2.1. Plant height (cm)**

Plant height was significantly influenced by the interaction between Azolla dry biomass and inorganic nitrogen fertilizer across both soil types ( $p \leq 0.01$ ) (Table 3.2). In soils collected from Hawassa Zuria and Wondo Genet, the greatest plant heights were recorded under the integrated application of 75 g Azolla  $\text{pot}^{-1}$  and 0.69 g N  $\text{pot}^{-1}$ , reaching 117.67 cm and 119.34 cm, respectively (Table 3.3). These values represent increases of 57.51% and 41.38% relative to the control treatments (Table 3.2). The observed enhancement in plant height can be attributed to the immediate availability of macro- and micronutrients supplied by the combined fertilizer sources. The readily accessible nitrogen from both organic and inorganic inputs likely stimulated vegetative growth, supporting stem elongation and overall biomass accumulation. This growth response is consistent with the well-established role of nitrogen in promoting cell expansion, elongation, and division, thereby fostering optimal vegetative development. Taranet et al. (2017) reported similar outcomes, demonstrating that the combined application of organic and inorganic fertilizers enhanced plant height by promoting cellular growth processes. Azolla serves as a valuable biofertilizer due to its high nitrogen content and capacity to supplement or partially replace synthetic nitrogen fertilizers (Zelege et al., 2024). Furthermore, Al-Bdairi and Kamal (2021) noted that the integration of Azolla and nitrogen fertilizers exerted a significant positive effect on plant height. Bio-organic fertilizers also support plant development during both vegetative and reproductive phases, thereby improving overall plant performance (Yu et al., 2022). Consistent results were reported by Adekiya et al. (2022), who observed that the combined use of organic and inorganic fertilizers enhanced the growth and yield of cucumber and tomato. Jumadi et al. (2014) further demonstrated that Azolla application increased the height of upland kangkong plants. The beneficial effects of Azolla are further linked to its contributions of available carbon and nitrogen, which enhance soil fertility (Vano et al., 2011), and its ability to stimulate enzyme activity and microbial biomass within the soil (Rose et al., 2006). Consequently, Azolla promotes microbial activity, accelerates nutrient recycling, and improves organic matter decomposition, thereby creating a more favorable soil environment for plant growth.

### **3.3.2.3. Number of fruits per cluster**

The interaction between Azolla dry biomass and inorganic nitrogen exerted a statistically significant effect ( $p < 0.05$ ) on the number of fruits per cluster at soil collected from both experimental locations (Table 3.2). The combined application of 75 g Azolla  $\text{pot}^{-1}$  and 0.69 g N  $\text{pot}^{-1}$  yielded the highest fruit numbers per cluster in Hawassa Zuria and Wondo Genet, with mean values of 4.00 and 4.34, respectively (Table 3.3). These outcomes correspond to increases of 66.5% and 76.1% relative to the untreated control (Table 3.2). Consistent trends were observed across both Hawassa Zuria and Wondo Genet sites, where the integrated application of Azolla dry biomass and inorganic nitrogen significantly ( $p < 0.05$ ) enhanced fruit cluster formation. Compared with the control treatments, the combined input of 75 g Azolla and 0.69 g N per pot increased the number of fruits per cluster by 66.5% in Hawassa Zuria and 76.1% in Wondo Genet. However, the higher number of fruits per cluster observed in tomatoes grown on soil collected from Wondo Genet, compared to those grown on soil collected from Hawassa Zuria, can be explained by several soil physicochemical advantages inherent to Andosols (Wondo Genet soils). Wondo Genet soils are typically derived from volcanic parent material and are characterized by high organic matter content, superior cation exchange capacity (CEC), and favorable moisture-retention properties. These attributes enhance nutrient availability and support sustained mineral uptake throughout the reproductive phase, thereby promoting improved flower development, pollination success, and subsequent fruit set (Leceta et al., 2024). Comparable results were reported by Zulkurnia et al. (2024), who demonstrated that Azolla application significantly improved fruit yield per plant. This enhancement is likely attributable to increased floral initiation and higher pollination success, culminating in a greater proportion of flowers developing into fruits.

### **3.3.3. Number of branches per plant**

In both collected soils, the analysis of variance result revealed that the interaction effects of Azolla (an organic) dry biomass and nitrogen significantly ( $P \leq 0.01$ ) influenced the number of branches. In contrast, the interaction effect demonstrated a significant ( $P \leq 0.01$ ) impact on the number of leaves per plant (Table 3.2). In the soils collected from the Hawassa Zuria and Wondo Genet locations, the treatment with combined applications of 75 g  $\text{pot}^{-1}$  of Azolla dry biomass with 0.69 g N  $\text{pot}^{-1}$  resulted in a maximum number of branches per plant (13.67 and 12.67) compared to the control group, respectively (Table 3.2). The data in Table 3.3 for the Hawassa Zuria and Wondo Genet areas showed that the combined application of nitrogen (0.69 g N  $\text{pot}^{-1}$ )

with Azolla dry biomass ( $75\text{ g pot}^{-1}$ ) increased the number of branches by 78.05% and 44.75%, respectively, compared to the control.

Nitrogen input strengthened the tomato branch, possibly due to beneficial encouragement of meristematic growth and new branches and leaves. Similarly, Rao et al. (2014) discovered that raising nitrogen levels improved chloroplast function, resulting in increased plant growth. Azolla, when used as an organic fertilizer, has a substantial impact on the number of tomato plant branches. The highest primary branch values were found at the highest level of Azolla and the lowest at the control plot. Similar findings were published by Sarolkar (2022), who discovered that while Azolla and inorganic N can both affect the number of branches on tomato plants, their effects may differ. Azolla compost can promote plant growth due to its nitrogen-fixing qualities and other nutrients, potentially leading to more branches. The effects of inorganic N can differ depending on the quantity used, even though it directly supplies nitrogen. Azolla contains a variety of macronutrients that encourage cell proliferation and elongation, increasing the number of tomato plants that branch. Azolla's high macro and micronutrient content makes nutrients more accessible in the root zone, improving plant uptake and causing tomato plants to branch (Zelege et al., 2024).

#### **3.3.2. 4. Fruit diameter (mm)**

Across the two soil collected locations, fruit diameter was significantly affected by the integrated application of Azolla dry biomass and inorganic nitrogen fertilizer ( $p \leq 0.01$ ). In soils collected from Hawassa Zuria and Wondo Genet, the combined treatment of  $75\text{ g Azolla dry biomass pot}^{-1}$  and  $0.69\text{ g N pot}^{-1}$  produced the largest fruit diameters, representing increases of 27.7% and 23.80% over the respective control treatments (Table 3.2). A progressive improvement in fruit diameter was observed as nutrient levels increased, with the highest response achieved under the integrated Azolla–inorganic N treatment. These findings suggest that the synergistic use of Azolla dry biomass with inorganic nitrogen confers greater benefits than the sole application of either input. The combined nutrient source likely enhances physiological efficiency and reproductive development, leading to improved fruit growth. This outcome aligns with the results reported by Zelege et al. (2024), who demonstrated that a high rate of Azolla integrated with inorganic nitrogen promotes the formation of more fruit clusters per plant, further supporting the advantage of integrated nutrient management in tomato cultivation

Table 3. 2. Interaction effects of Azolla dry biomass and inorganic N fertilizers on plant height, fruit number per cluster, number of branches, number of cluster per plant, and fruit diameter on tomato plants soil collected from Hawassa Zuria and Wondo Gnet areas.

Treatment combinations		Soil collected from Hawassa Zuria district					Soil collected from Wondo Genet district				
Azolla(g pot <sup>-1</sup> )	N (g pot <sup>-1</sup> )	PH	FrNC	NB	NCPP	FD	PH	FrNC	NB	NCPP	FD
0	0	50.00 <sup>i</sup>	1.34 <sup>h</sup>	3.00 <sup>i</sup>	2.34 <sup>hg</sup>	37.04 <sup>kl</sup>	69.67 <sup>h</sup>	1.04 <sup>i</sup>	7.00 <sup>ef</sup>	3.00 <sup>fg</sup>	38.00 <sup>jk</sup>
	0.23	59.00 <sup>h</sup>	1.68 <sup>gfh</sup>	6.00 <sup>fgh</sup>	4.00 <sup>dfge</sup>	37.46 <sup>kl</sup>	78.34 <sup>g</sup>	1.17 <sup>ih</sup>	9.00 <sup>cd</sup>	4.00 <sup>fge</sup>	40.93 <sup>ij</sup>
	0.46	74.67 <sup>g</sup>	1.67 <sup>gfh</sup>	10.00 <sup>bc</sup>	6.34 <sup>dc</sup>	45.41 <sup>ef</sup>	90.00 <sup>f</sup>	1.75 <sup>efg</sup>	5.34 <sup>fg</sup>	4.34 <sup>dfge</sup>	44.80 <sup>h</sup>
	0.69	78.67 <sup>gf</sup>	2.34 <sup>cfed</sup>	10.00 <sup>bc</sup>	9.34 <sup>ba</sup>	45.06 <sup>efg</sup>	98.67 <sup>d</sup>	2.49 <sup>dc</sup>	11.34 <sup>ab</sup>	9.67 <sup>a</sup>	47.23 <sup>cd</sup>
25	0	73.00 <sup>g</sup>	1.43 <sup>gh</sup>	7.00 <sup>efh</sup>	3.67 <sup>hfge</sup>	40.26 <sup>j</sup>	79.00 <sup>g</sup>	1.51 <sup>fhg</sup>	8.00 <sup>de</sup>	4.00 <sup>gfe</sup>	39.62 <sup>j</sup>
	0.23	87.00 <sup>ed</sup>	1.96 <sup>gfeh</sup>	8.34 <sup>de</sup>	4.00 <sup>dfge</sup>	44.94 <sup>fgh</sup>	87.34 <sup>d</sup> ef	1.99 <sup>fe</sup>	10.34 <sup>bc</sup>	6.67 <sup>bc</sup>	43.28 <sup>gh</sup>
	0.46	79.00 <sup>fg</sup>	2.81 <sup>cd</sup>	10.34 <sup>b</sup>	6.34 <sup>dc</sup>	45.96 <sup>ef</sup>	85.00 <sup>e</sup> f	1.76 <sup>feg</sup>	9.67 <sup>cd</sup>	6.34 <sup>dc</sup>	47.05 <sup>cd</sup>
	0.69	89.34 <sup>d</sup>	2.68 <sup>cbd</sup>	10.34 <sup>b</sup>	10 <sup>a</sup>	47.97 <sup>c</sup>	103.6 7 <sup>c</sup>	2.11 <sup>de</sup>	12.67 <sup>a</sup>	10.67 <sup>a</sup>	46.58 <sup>de</sup>
50	0	82.33 <sup>ef</sup>	2.15 <sup>cfed</sup>	2.67 <sup>ij</sup>	2.67 <sup>hfg</sup>	44.64 <sup>ghi</sup>	81.67 <sup>g</sup> f	1.42 <sup>ihg</sup>	8.34 <sup>de</sup>	5.00 <sup>dfce</sup>	43.08 <sup>gh</sup>
	0.23	85.67 <sup>ed</sup>	2.27 <sup>fed</sup>	7.67 <sup>de</sup>	5.00 <sup>dfce</sup>	47.39 <sup>cd</sup>	91.00 <sup>d</sup> e	1.96 <sup>fe</sup>	7.67 <sup>e</sup>	6.00 <sup>dce</sup>	47.21 <sup>cd</sup>
	0.46	97.67 <sup>c</sup>	3.05 <sup>c</sup>	6.00 <sup>fgh</sup>	6.67 <sup>c</sup>	46.27 <sup>de</sup>	99.34 <sup>c</sup>	2.03 <sup>de</sup>	8.34 <sup>de</sup>	5.67 <sup>dce</sup>	47.45 <sup>bc</sup>

	0.69	110.00 <sup>b</sup>	2.43 <sup>ced</sup>	10.00 <sup>bc</sup>	6.00 <sup>dce</sup>	49.87 <sup>c</sup>	104.3 4 <sup>c</sup>	2.70 <sup>c</sup>	11.00 <sup>ab</sup>	8.67 <sup>ba</sup>	48.43 <sup>ba</sup>
75	0	86.00 <sup>ed</sup>	3.06 <sup>b</sup>	3.00 <sup>i</sup>	1.34 <sup>h</sup>	43.46 <sup>i</sup>	88.67 <sup>d</sup> e	1.83 <sup>teg</sup>	5.34 <sup>fg</sup>	2.67 <sup>g</sup>	47.45 <sup>bc</sup>
	0.23	85.67 <sup>ed</sup>	3.07 <sup>c</sup>	6.00 <sup>fgh</sup>	5.00 <sup>dfce</sup>	47.97 <sup>c</sup>	92.34 <sup>d</sup> e	2.96 <sup>c</sup>	10.34 <sup>bc</sup>	4.34 <sup>dfge</sup>	48.26 <sup>ba</sup>
	0.46	102.00 <sup>c</sup>	3.15 <sup>b</sup>	8.67 <sup>cd</sup>	4.00 <sup>dfge</sup>	49.16 <sup>b</sup>	111.3 4 <sup>b</sup>	3.23 <sup>b</sup>	10.67 <sup>bc</sup>	5.00 <sup>dfce</sup>	45.79 <sup>ef</sup>
	0.69	117.67 <sup>a</sup>	4.00 <sup>a</sup>	13.67 <sup>a</sup>	5.00 <sup>dfce</sup>	51.23 <sup>a</sup>	119.3 4 <sup>a</sup>	4.34 <sup>a</sup>	12.67 <sup>a</sup>	5.67 <sup>dce</sup>	49.87 <sup>a</sup>
	<b>LSD (5%)</b>	<b>6.36</b>	<b>0.72</b>	<b>1.40</b>	<b>2.46</b>	<b>1.21</b>	<b>5.93</b>	<b>0.47</b>	<b>2.18</b>	<b>2.13</b>	<b>2.28</b>
	<b>CV (%)</b>	<b>4.50</b>	<b>8.74</b>	<b>7.49</b>	<b>7.84</b>	<b>5.56</b>	<b>3.91</b>	<b>2.39</b>	<b>6.33</b>	<b>7.62</b>	<b>7.12</b>

Means followed by the same letter(s) in the same column are not significantly different at the 5 % level of significance. PH= plant height, FrNC = number of fruit per cluster; NB = number of branches per plant; NCPP = number of clusters per plant; FD= fruit diameter.

### **3.3.3. Effects of Azolla dry biomass and inorganic N fertilizers on marketable and total fruit yield of tomato**

#### **3.3.3.1. Marketable fruit yield of tomato**

Across both soil collection sites, the application of Azolla dry biomass in combination with inorganic nitrogen fertilizer significantly increased marketable fruit yield ( $p < 0.01$ ). The integrated treatment of  $0.69 \text{ g N pot}^{-1}$  and  $75 \text{ g Azolla dry biomass pot}^{-1}$  produced the highest marketable tomato yields in soils from Hawassa Zuria and Wondo Genet, with values of  $1088.43 \text{ g plant}^{-1}$  and  $833.73 \text{ g plant}^{-1}$ , respectively (Figure 3.1a and b). In contrast, the control treatment resulted in the lowest marketable yields at both locations, with yield improvements of 81.26% and 75.77%, respectively, under the combined fertilizer treatment. The maximum performance of the Hawassa Zuria soil, characterized as sandy loam, can be attributed to its favorable physical properties. Sandy loam offers a more optimal balance of aeration, drainage, and root penetration compared to the sandy clay loam soil collected from Wondo Genet (Ger et al., 2024). The higher sand fraction in sandy loam enhances soil porosity and reduces mechanical resistance to root growth, thereby improving root system development, nutrient uptake efficiency, and soil water dynamics (Nega et al., 2025). As a result, sandy loam promotes more favorable root–soil interactions and physiological responses, leading to greater tomato yield and improved fruit quality under pot cultivation conditions (Baroutkoob et al., 2024). The positive yield response associated with the combined use of organic and inorganic fertilizers may also be linked to enhanced root development and the stimulation of plant growth regulators, as evidenced by improvements in yield-related traits such as fruit diameter. Similar trends have been documented in previous studies. Mallika et al. (2022) reported yield improvement following integrated fertilization. Yang et al. (2020) demonstrated that incorporating Azolla with urease inhibitors mitigates ammonia volatilization, increases nitrogen-use efficiency, and subsequently enhances rice grain yield. Bipradas et al. (2016) showed that tomato yield was maximized when 50% organic and 50% inorganic fertilizer were applied. Warner et al. (2004) found that nitrogen fertilization increased fruit yield up to  $250 \text{ kg N ha}^{-1}$ , while Ren et al. (2010) reported that nitrogen inputs as high as  $645 \text{ kg N ha}^{-1}$  boosted marketable tomato yield. Similarly, mixed fertilizer application outperformed single-source fertilization in eggplant (Ullah et al., 2008) and cabbage (Golam, 2015). Azolla contributes additional agronomic benefits by gradually releasing nitrogen that plants can absorb to increase biomass and grain production (Guo et al., 2019). Moreover, Azolla promotes microbial activity, enhances soil quality, and improves nutrient availability (Zhang et al., 2020). Nitrogen itself is essential for fruit development, as it supports vegetative growth, reproductive processes, and protein synthesis, ultimately contributing to increased fruit weight (Reddy et al., 2002).

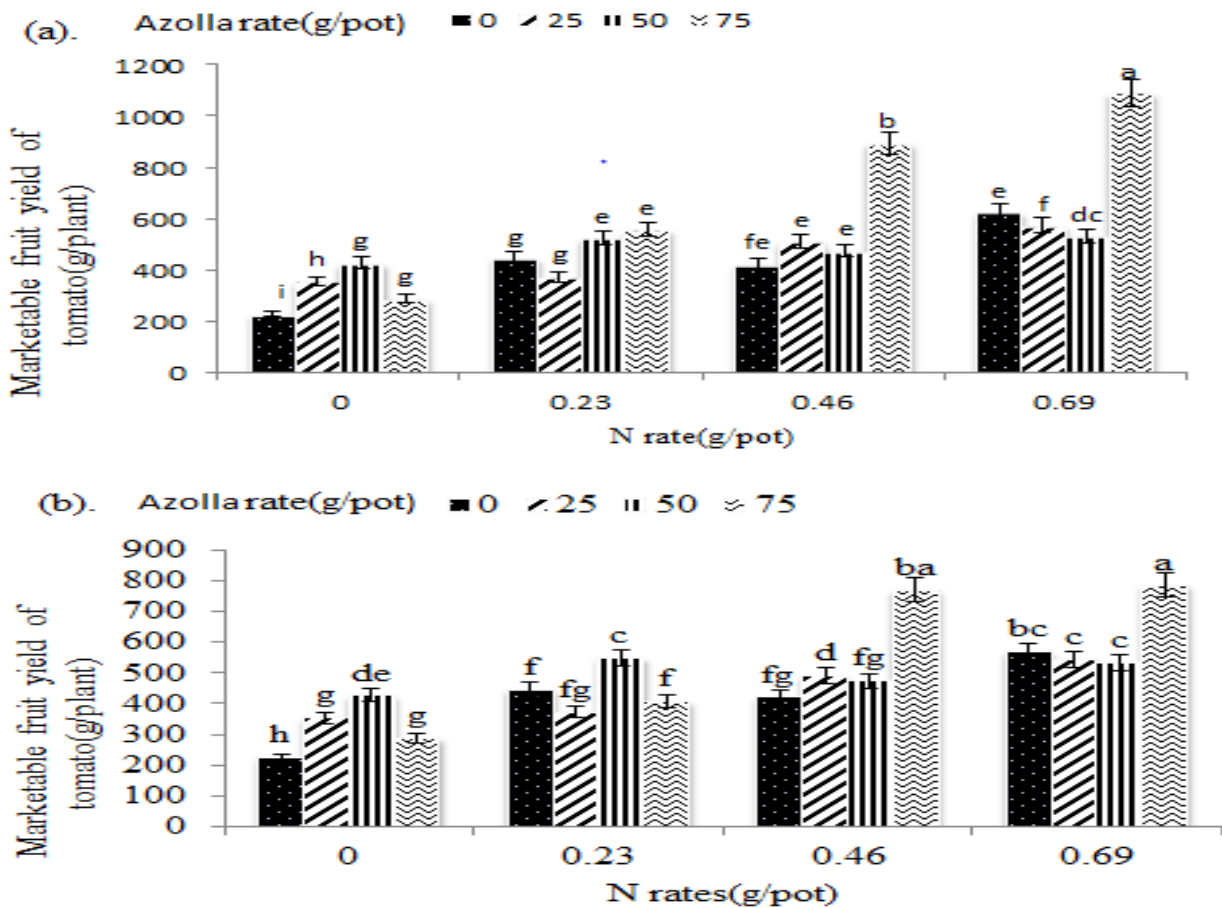


Figure 3. 1. The interaction effects of Azolla dry biomass and inorganic N fertilizers on marketable fruit yield of tomato in (a) Hawassa Zuria and (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $p < 0.05$  (LSD).

### 3.3.3.2. Total fruit yield

In both soil collected locations, it was demonstrated that the total fruit yield is significantly affected ( $P < 0.01$ ) by the interaction application of nitrogen and Azolla fertilizers (Figures 3.2a and b). Azolla and nitrogen fertilizer can improve fruit development and yield when used sparingly. The interaction of  $0.69 \text{ g N pot}^{-1}$  and  $75 \text{ g Azolla pot}^{-1}$  resulted in the highest total fruit yields of  $1368.50 \text{ g/plant}$  and  $1136.22 \text{ g/plant}$ , respectively, the soil collected from Hawassa Zuria and Wondo Genet (Figures 3.2a and b). The control group produced the fewest fruits, weighing  $241.60$  and  $246.21 \text{ g/plant}$ , respectively. The total fruit output increased by  $82.35\%$  and  $78.33\%$ , respectively, as compared to the control group. The higher overall fruit output of tomatoes caused by the combined application of Azolla and N fertilizers may be due to the tomato plants' increased nitrogen availability from Azolla and inorganic N fertilizer. Combining Azolla with inorganic nitrogen fertilizer can increase tomato fruit yield by promoting photosynthesis, fruit development, and vegetative growth through a well-balanced nutrient

supply. Azolla improves soil properties and slowly releases nitrogen, whereas inorganic fertilizers increase nutrient availability. The low yield seen in Azolla unincorporated treatments could be attributed to a slower rate of decomposition and probably reduced availability of Azolla-N to rice plants. These results are comparable with other studies by Singh et al. (2018) and Mahmud et al. (2016), which discovered that incorporating dry Azolla biomass into the soil regularly raised rice grain production. This conclusion is also in line with the results of Meena et al. (2015), who found that using organic manures along with the recommended quantity of inorganic fertilizer enhanced tomato production overall. Tomato yield is significantly impacted when Azolla and inorganic nitrogen fertilizer are applied together. To successfully and efficiently produce vast quantities of high-quality fruits, the tomato's nutritional needs must be carefully taken into account (Biramo et al., 2019).

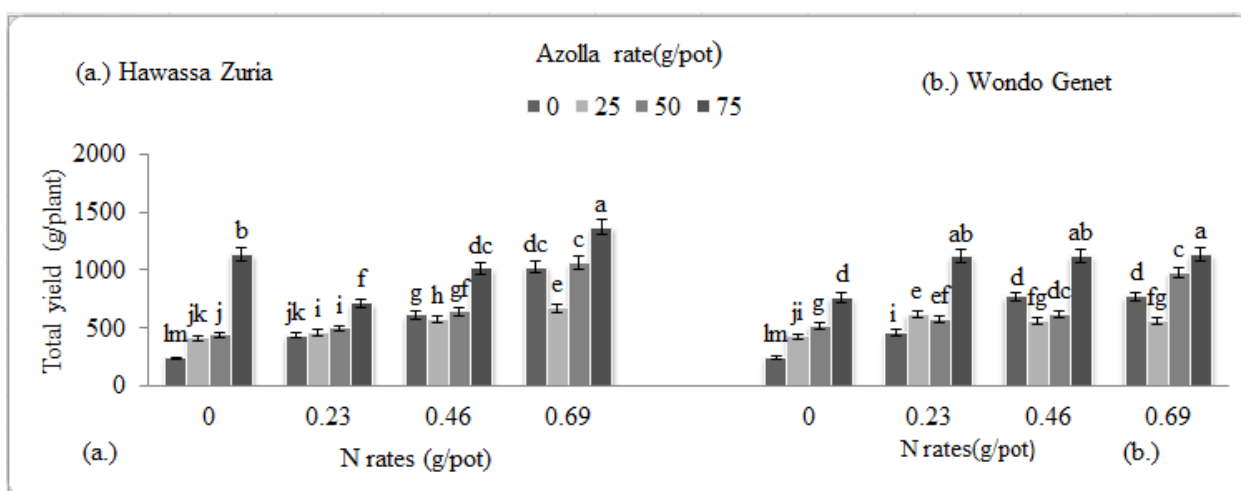


Figure 3.2. The interaction effects of Azolla dry biomass and inorganic N fertilizers on total fruit yield of tomato in (a) Hawassa Zuria and (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

### 3.4. Conclusions

The study observed that the integrated application of Azolla dry biomass and inorganic nitrogen fertilizers affected the soil chemical properties and yield of tomatoes. The results showed significant improvements in both soil chemical properties and tomato fruit yield. Soil collected from Hawassa Zuria, the interaction effect of Azolla and inorganic nitrogen fertilizer rates had a significant impact on soil pH, because the soil pH is reduced due to Azolla biomass decomposition, it adds organic matter to the soil. The soil collected from Hawassa Zuria and Wondo Genet locations, the highest values of CEC were recorded from the combined application of  $75 \text{ g pot}^{-1}$  Azolla dry biomass with  $0.69 \text{ g N pot}^{-1}$ , with values of  $29.50 \text{ cmol}(+) / \text{kg}$  and  $37.37 \text{ cmol}(+) / \text{kg}$ ,

respectively, compared to control treatments. The interaction of Azolla dry biomass as a source of organic and inorganic nitrogen fertilizers improved soil fertility and crop productivity in both collected soils. Specifically, the combined application of 75 g pot<sup>-1</sup> of Azolla dry biomass and 0.69 g pot<sup>-1</sup> of N resulted in these improvements. This technique not only enhances soil quality and crop yield but also helps mitigate the harmful environmental effects of traditional inorganic fertilizers. As a result, combining Azolla dry biomass and inorganic N fertilizers could be an appropriate fertilizing strategy for tomato growing, particularly in collected soil types with difficult soil conditions. Future research should test this Azolla dry biomass with inorganic N fertilizer in a field experiment to confirm the results.

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## CHAPTER FOUR

### 4. ENHANCING SOIL FERTILITY, NITROGEN USE EFFICIENCY AND TOMATO PRODUCTIVITY THROUGH INTEGRATED USE OF AZOLLA DRY BIOMASS AND NITROGEN FERTILIZERS UNDER IRRIGATED CONDITIONS IN HAWASSA ZURIA AND WONDO GENET, SOUTHERN ETHIOPIA

#### Abstract

*Low soil fertility and reduced agricultural productivity are the main challenges of Ethiopian farmers. To maintain soil fertility and increase crop yields under irrigated conditions, it is necessary to use both organic and inorganic fertilizer sources efficiently. In this context, combining the biomass of organic inputs like Azolla, a nitrogen-fixing aquatic fern, with inorganic nitrogen fertilizers is a promising technique for improving soil health and crop yield. However, only a few studies have looked into the combined impacts of Azolla dry biomass and inorganic nitrogen on soil nutrient dynamics and crop performance in irrigated fields. As a result, a two-year field experiment (2022/23 and 2023/24) was conducted in the Hawassa Zuria and Wondo Genet districts of Sidama Region to assess the combined impacts of Azolla dry biomass and inorganic nitrogen fertilizer on soil and crop productivity under irrigation. The study specifically aimed to (i) determine the effects of Azolla dry biomass as an organic nitrogen source on soil chemical properties and tomato production, (ii) determine the interaction effects of Azolla dry biomass and inorganic nitrogen on tomato yield and yield components, N uptake, and N use efficiency in tomato production, and (iii) identify the optimum combination of Azolla dry biomass and N fertilizers for maximizing tomato productivity. The experiment included four levels of Azolla dry biomass (0, 5, 10, and 15 t ha<sup>-1</sup>) and four levels of inorganic nitrogen (0, 46, 92, and 138 kg N ha<sup>-1</sup>) arranged in a factorial combination using a randomized complete block design (RCBD) with three replications. Soil samples were analyzed both before and after planting. Azolla dry biomass and inorganic N rates significantly affected most parameters in both locations ( $P < 0.01$ ). In Hawassa Zuria and Wondo Genet, applying Azolla dry biomass (15 t ha<sup>-1</sup>) and nitrogen (138 kg ha<sup>-1</sup>) increased soil total nitrogen levels (0.34% and 0.44%), organic carbon content (4.25% and 5.08%), available P (17.97 mg kg<sup>-1</sup> and 35.38 mg kg<sup>-1</sup>), and cation exchange capacity (28.57 cmol(+) kg<sup>-1</sup> and 35.49 cmol(+) kg<sup>-1</sup>) compared to the control group. Furthermore, the results showed that using Azolla dry biomass and N fertilizer had a significant impact on tomato growth and yield. Hawassa Zuria and Wondo Genet produced the highest marketable fruit, with 23.76 and 26.35 t ha<sup>-1</sup>, respectively. This was accomplished by using 15 t ha<sup>-1</sup> of Azolla dry biomass and 138 kg ha<sup>-1</sup> of nitrogen. The combined application of Azolla dry biomass 15 t ha<sup>-1</sup> and 138 kg N ha<sup>-1</sup> led to the highest fruit N uptake (1093.48 kg ha<sup>-1</sup> in Hawassa Zuria and 1486.94 kg ha<sup>-1</sup> in Wondo Genet), while the control treatment had the lowest. Based on the findings of this trial, we concluded that the combination of Azolla dry biomass and inorganic N fertilizer improved soil chemical properties like organic carbon, total nitrogen, cation exchange capacity and Soil pH and available P, resulting in greater nitrogen uptake and long-term tomato production in the study locations. Overall, the integrated*

*application of 15 t ha<sup>-1</sup> Azolla dry biomass and 138 kg N ha<sup>-1</sup> is recommended at both the two tested sites and similar agro-ecological environments for improved soil fertility and productivity of tomato.*

**Keywords:** Crop productivity; declining soil fertility, improve soil properties; soil pH; organic matter ; total nitrogen

#### **4.1. Introduction**

Soil fertility decline is a major issue in Ethiopian agriculture, as a large proportion of the population (85%) relies on it (Yebo, 2015). However, this population is not able to achieve reliable production from agriculture, leading to a decline in crop yields that result in poverty and malnutrition (Prosekov and Inavona, 2016). Studies have shown that in some parts of Ethiopia (Yebo, 2015), the major problems contributing to soil fertility decline include depletion of soil nutrients, excessive use of chemical fertilizers, reduction in soil organic matter, soil erosion, crop harvest removal, and inadequate fertilizer applications. Many of the same chemical and physical soil properties that are affected by onsite soil degrading processes also impact soil fertility and nutrient availability to plants (Havlin and Heiniger, 2020). Understanding these processes and interactions is essential for optimizing plant nutrient availability and minimizing nutrient losses to the environment (Briat et al., 2020). When plants are removed from a field or soil sediments are transported offsite, the nutrients in the soil become depleted (Liu and Yu, 2020). Soil nutrient supply relies on the soil's ability to buffer nutrient loss through crop removal (Schröder et al., 2016). Therefore, to optimize nutrient supply to crops and minimize the environmental risk of nutrient use, it is crucial to understand nutrient reactions and processes in soils (soil fertility) and efficiently manage inorganic and organic nutrient inputs (nutrient management) to ensure an adequate soil nutrient supply (Raza et al., 2023). Agricultural producers must take advantage of soil- and plant-management technologies that increase plant productivity and minimize soil productivity loss through runoff, leaching, and nutrient depletion (Havlin and Heiniger, 2020).

Tomato ((*Solanum Lycopersicon* L.) is a nutritionally and economically significant horticultural crop, providing key vitamins, minerals, and antioxidants (Tsfay et al., 2018). In Ethiopia, it ranks as the fourth most cultivated vegetable, accounting for 5.45% of vegetable production and 4.49% of the total vegetable production area (Abera et al., 2020). Despite its importance, national

tomato yields average only 8 t ha<sup>-1</sup>, far below the global mean of 34 t ha<sup>-1</sup>, with production declining as cultivation area decreased from 6,298.63 ha in 2016/17 to 5,235.19 ha in 2017/18 (Beyene and Mulu, 2019). This yield gap is primarily driven by soil fertility depletion, insufficient and inefficient fertilizer use, and imbalanced nutrient management, which intensify nutrient deficiencies and diminish soil productivity, particularly in key tomato-growing areas such as Sidama (Tesfay et al., 2018). To enhance tomato growth and productivity under Ethiopian conditions, optimizing nitrogen fertilizer rates is crucial (Beyene and Mulu, 2019).

Global fertilizer demand is projected to increase by approximately 70% by 2050, highlighting the continued dependence on inorganic nutrient inputs (Akhtar et al., 2021). However, insufficient nitrogen (N) fertilizer application on soil results in disturbing the soil nutrient balance; depletion of organic matter reduces water and nutrient uptake by crops (Razavipour et al., 2018; Song et al., 2022). Additionally, the inadequate application of N fertilizers on tomatoes results in reduced yield (Zelege et al., 2024). These challenges emphasize the importance of integrating organic fertilization to restore soil quality, provide a balanced nutrient supply, and promote sustainable crop growth (Fawzy et al., 2016).

Organic fertilizers, rich in organic matter and diverse nutrients, improve soil quality and nutrient availability, thereby supporting sustainable agricultural intensification and stable yields (Ning et al., 2017; Zhu et al., 2019; Seleiman et al., 2020). They stimulate soil microbial communities, increase soil organic matter, and enhance nutrient uptake efficiency, collectively improving soil physicochemical properties and crop performance (Kimani et al., 2021; Novair et al., 2020; Razavipour et al., 2018). Among these amendments, Azolla is notable for its biological nitrogen fixation capacity of 30–100 kg N ha<sup>-1</sup> per crop cycle and its suitability for wet environments, making it valuable in organic and low-input systems (Kassem and El-Aal, 2016; Kamaruddin et al., 2019; Abou Hussien et al., 2020; Elsebaie et al., 2022). Integrating organic and inorganic nitrogen sources further enhances soil health, nutrient availability, and overall agricultural productivity (Bayu, 2020).

Azolla application significantly reduces nitrogen losses by 26–48% and increases nitrogen recovery efficiency by 49–64% due to its rapid decomposition and release of bioavailable nitrogen (Thapa and Poudel, 2021). It improves nitrogen use efficiency, crop yield, and essential

soil properties, including total nitrogen, organic matter, available phosphorus, and cation exchange capacity. Evidence from tropical agroecosystems indicates that Azolla enhances productivity while remaining economically and environmentally sustainable (Bhuvaneshwari & Kumar, 2013). However, field-based research on Azolla's impacts on soil properties and crop performance remains limited in Ethiopia (Feyisa et al., 2013).

There is limited information on the combined use of Azolla dry biomass and inorganic N on tomato fruit yield and growth, as well as their contribution to enhancing soil chemical characteristics in the research locations. Furthermore, tomatoes are a popular vegetable crop in the Hawassa Zuria and Wondo Genet districts; however, the integrated use of Azolla dry biomass and inorganic N fertilizer is not yet fully implemented. Based on these insights, this research aims to evaluate the effects of integrated Azolla dry biomass and inorganic N fertilizer applications on soil chemical properties and tomato yield. The study also seeks to determine the combined application of Azolla and N fertilizer on chemical soil properties; nitrogen uptake and nitrogen use efficiency in tomato production and identify the optimal combination of Azolla dry biomass and nitrogen fertilizers for maximizing tomato productivity.

## **4.2. Materials and Methods**

### **4.2.1 Description of study areas**

The study areas are as mentioned in section 2.2.1.

### **4.2.2. Experimental design, treatments, and procedures**

The field experiments were conducted over two consecutive irrigation seasons (2022/23 and 2023/24) in two selected districts of the Sidama Region, southern Ethiopia. A factorial combination of four rates of dry Azolla biomass (0, 5, 10, and 15 t ha<sup>-1</sup>) as an organic nitrogen source and four levels of inorganic nitrogen fertilizer (0, 46, 92, and 138 kg N ha<sup>-1</sup>) was evaluated, resulting in 16 treatment combinations. Treatments were arranged in a randomized complete block design (RCBD) with three replications, having 48 total plots per location. The improved tomato variety 'Gelilema' was used in this study. Seedlings were raised in a 5 m × 1 m seedbed near the experimental site to minimize transplanting shock. Transplanting was performed four weeks after sowing when seedlings reached 15–25 cm in height, at a spacing of 100 cm between rows and 30 cm between plants within rows.

Prior to transplanting, all plots were uniformly prepared using oxen plowing to a depth of 20 cm, followed by harrowing to approximately 10 cm using conventional tillage. Each plot measured 12 m<sup>2</sup> (4 m × 3 m), with 1.5 m spacing between blocks and 1 m between plots. The number of rows per plot was four, and the number of plants per row was nine. Phosphorus fertilizer, in the form of triple superphosphate (TSP), was applied fully at transplanting, while nitrogen (urea used as a source of N) was applied in two equal splits: half the dose at transplanting and the remaining half at 35 days after transplanting. Azolla biomass was sourced from the School of Animal and Range Sciences, Hawassa College of Agriculture. The moisture content of Azolla biomass was 75%. It was gently washed, dried, and analyzed for dry weight as well as total nitrogen content. The dried Azolla was incorporated into the soil 15 days before transplanting via broadcasting application, excluding the control plots.

The recommended agronomic management practices were carried out throughout the different growth stages of the crop. Ridges were prepared with 100 cm distances between rows using an oxen-mounted rigger, and seedlings were transplanted to the permanent experimental field at a spacing of 0.30 m \* 1 m, as recommended by Lemma (2002). The tomato field was irrigated using the furrow method at intervals of five to six days, depending on the prevailing weather conditions throughout the crop cycle (Edossa Etissa et al., 2013).

#### **4.2.3. Soil Sampling**

Before the experiment, a composite surface soil sample (0–20 cm depth) was collected from each experimental site of the selected kebeles and analyzed for baseline soil properties, including soil pH (H<sub>2</sub>O), soil texture, available phosphorus, total nitrogen (TN), cation exchange capacity (CEC), and organic carbon (OC). After harvest, additional plot-wise surface soil samples (0–20 cm) were collected to assess the effects of Azolla application on soil pH, electrical conductivity (EC), TN, OC, Ava.P, and CEC. All samples were taken to the soil science laboratory for analysis of selected chemical properties. The soils were air-dried, crushed with a mortar and pestle, and sieved through a 2 mm mesh. For total nitrogen (TN) and organic carbon (OC) analysis, subsamples were further sieved through a 0.5 mm mesh.

#### **4.2.4. Soil Analysis**

Particle size distribution (soil texture) was determined using the modified Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was measured potentiometrically with a combined glass

electrode pH meter in a 1:2.5 soil-to-water suspension (Van Reeuwijk, 1992). Electrical conductivity (EC) was measured using a conductivity meter in a soil–water extract (Okalebo et al., 2002). Cation exchange capacity (CEC) was determined by saturating the soil with 1 M  $\text{NH}_4\text{OAc}$  (pH 7), washing with ethanol, and then displacing adsorbed ammonium with sodium ions (Chapman et al., 1961). The released ammonium in the distillate was back-titrated with dilute acid, and the CEC was expressed as the amount of ammonium exchanged. Organic carbon (OC) content was measured by the wet digestion chromic acid method described by Walkley and Black (1934). Total nitrogen was determined using the Kjeldahl digestion procedure as outlined by Jackson (1958). Available phosphorus (Ava.P) was extracted following the standard Olsen method (Olsen et al., 1954).

#### **4.2.5. Azolla sampling and analysis**

Before incorporating Azolla dry biomass into the soil as an organic source of nitrogen, its nitrogen content was analyzed. Samples were analyzed in the laboratory for pH, nitrogen, phosphorus, CEC, and organic carbon. Total nitrogen in the Azolla was estimated using the micro Kjeldahl method (Jackson, 1962), with a 10 g oven-dried sample digested and used for analysis. Total N, phosphorus, pH, cation exchange capacity (CEC), and organic carbon were determined following standard soil analysis procedures.

#### **4.2.6. Plant nitrogen analysis**

To measure nitrogen (N) content, plants were clipped at their base during the maturity stage. A total of 500 g of shoot and fruit samples were collected, oven-dried at 65°C for 72 hours, and then ground to pass through a 1 mm mesh sieve for N concentration. The total N was analyzed using the Kjeldahl method (Jackson, 1962).

#### **4.2.7. Calculation of plant nitrogen uptakes and use efficiencies**

The fruit nitrogen uptake (FNU) and shoot nitrogen uptake (SNU) were calculated by multiplying fruit yield and shoot yield (without fruit) with the respective N concentration and then dividing by 100.

Total N uptake/ha = Fruit yield/ha x % N in the fruit + shoot/ha x % N in the shoot without fruit.

The decomposition of Azolla biomass releases a large amount of nitrogen into soil for plant absorption, 75–80% of the total collected (Korsa et al., 2024). For the present study, 75% decomposition of Azolla biomass was used to release nutrients for plant absorption.

#### 4.2.7.1. Computation of N-agronomic efficiency, physiological use efficiency, and N-apparent recovery

The use efficiency of applied nitrogen was worked out in terms of agronomic efficiency (AE), agro physiological use efficiency (APE), and apparent recovery (AR) by employing the following formula used by Dua *et al.* (2009) and Sharma and Banik (2012).

Agronomic efficiency (AE) was calculated using the following equation (Eq.):

$$AE = \frac{Y_f - Y_c}{N_a} \dots\dots\dots \text{Eq. (4.1)}$$

Where AE = agronomic efficiency;

Y<sub>f</sub> = fruit yield (kg ha<sup>-1</sup>) from fertilized

Y<sub>c</sub> = fruit yield (kg ha<sup>-1</sup>) from the control plot and

N<sub>a</sub> = amount of N applied (kg ha<sup>-1</sup>).

Agro Physiological use efficiency (APE) was calculated using the following equation formula (Baligar *et al.*, 2001):

$$APE (\%) = \frac{Y_f - Y_c}{N_{upf} - N_{upc}} \dots\dots\dots \text{Eq. (4.2)}$$

APE (fruits kg/kg N applied) = (Y<sub>f</sub> – Y<sub>c</sub>) / (N<sub>upf</sub> – N<sub>upc</sub>)

Where: Y<sub>f</sub> = fruit yield (kg ha<sup>-1</sup>) from a fertilized plot;

Y<sub>c</sub> = fruit yield (kg ha<sup>-1</sup>) from the control plot;

N<sub>upf</sub> = nitrogen uptake (kg ha<sup>-1</sup>) in a fertilized plot and

N<sub>upc</sub> = nitrogen uptake (kg ha<sup>-1</sup>) in the control plot.

Apparent recovery AR (%) was calculated as follows:

$$AR (\%) = \frac{N_{upf} - N_{upc}}{N_a} \dots\dots\dots \text{Eq. (4.3)}$$

Where AR = apparent recovery;

Nupf = nitrogen uptake ( $\text{kg ha}^{-1}$ ) in a fertilized plot;

Nupc = nitrogen uptake ( $\text{kg ha}^{-1}$ ) in the control plot and

Na = amount of N applied ( $\text{kg ha}^{-1}$ ).

#### **4.2.8. Data Collection**

The tomato field data collected included plant height, number of branches per plant, number of flower clusters, number of fruit clusters per plant, and fruit number per cluster. This information's were determined from five randomly selected representative plants per plot. Additionally, the average fruit weight per plant (kg), marketable fruit weight ( $\text{t ha}^{-1}$ ), and fruit yield per hectare ( $\text{t ha}^{-1}$ ) were recorded, along with other agronomic characteristics of tomato plants for each treatment. The necessary nitrogen use efficiency data were collected, including N shoots (N content% and N uptake), fruit N (N content% and N uptake), and total N uptake of tomato, NUE, shoot N content, shoot biomass, and tomato fruit yield. The data were collected by randomly selecting five plants from the two central rows of each plot, excluding the border rows and border plants. At the end of the season, the amount of fruit yield produced was harvested and weighed from each plot.

#### **4.2.9. Data Analysis**

The soil and agronomic data analyzed data were tested for homogeneity of error variance using Bartlett's test (Gomez and Gomez, 1984). Accordingly, data were subjected to combined analysis of variance over years of each location (2022/23 and 2023/24) using mixed effects model whereas Azolla dry biomass and nitrogen were assigned as fixed factors, year, location and replications were considered random factors. Means separation was performed using the least significant difference (LSD) test at a 5% significance level.

### **4.3. Results and Discussion**

#### **4.3.1. Physicochemical properties of soils before planting**

The results of soil physicochemical analysis before planting are presented in Table 4.1 for both locations. In Hawassa Zuria and Wondo Genet locations, the soil textural class was sandy loam and sandy clay loam, respectively (Table 4.1). The results from Hawassa Zuria and Wondo Genet showed that the soil had a strongly alkaline and neutral reaction, with pH values of 8.59 and 7.25, total nitrogen levels of 0.16% and 0.30%, available phosphorus levels of 8.95 and 30.62  $\text{mg kg}^{-1}$ ,

organic carbon levels of 2.21% and 3.19%, C/N ratios of 13.81 and 11.40, and cation exchange capacities (CEC) of 24.22 and 28.25 cmol (+) kg<sup>-1</sup>, respectively, before planting (Table 4.1). According to Landon (1991), rating system was used to categorize total nitrogen as low (<0.2), medium (0.2-0.5), and high (>0.5). The total nitrogen of the soils before planting was low in Hawassa Zuria and medium in Wondo Genet location. Olsen et al. (1954) classified available phosphorus content as low (<5 mg kg<sup>-1</sup>), medium (5–10 mg kg<sup>-1</sup>), and high (>10 mg kg<sup>-1</sup>). The available phosphorus levels in the soil before planting fell within the medium range in Hawassa Zuria and very high in Wondo Genet location. CEC ranges of 5–15, 15–25, and 25–40 cmol (+) kg<sup>-1</sup> are considered low, medium, and high, respectively. Overall, the nutrient content of the study locations was good in terms of major plant nutrients availability. However, the strong alkalinity of the soil may lead to the sorption of available phosphorus, as it has the ability to reduce soil pH by increasing organic acid levels. Therefore, the application of organic matter is essential to neutralize the soil solution.

Table 4. 1. Physicochemical properties of the soil before planting in the study areas

		Districts	
		Hawassa Zuria	Wondo Genet
Soil properties	Units		
Particle size distribution			
Sand	%	76	54

Clay	%	10	25
Silt	%	14	21
Textural class	–	Sandy loam	Sandy clay loam
Chemical properties			
pH(H <sub>2</sub> O,1:2.5)	-	8.64	7.25
EC	dS/m	3.15	1.52
Organic carbon	%	2.21	3.19
Total nitrogen	%	0.16	0.28
C/N	-	13.81	11.40
Ava.P	mg kg <sup>-1</sup>	8.95	30.62
CEC	(cmol(+) kg <sup>-1</sup> )	24.22	28.25

#### 4.3.2. Chemical properties of Azolla dry biomass before incorporation

The chemical properties of Azolla before planting were presented in Table 4.2. The analysis results showed that the pH, TN, OC,C/N, Ava.p and CEC were 7.02 (neutral), 5.08%, 48%, 9.45, 276.40 mg kg<sup>-1</sup>, and 41.35 cmol(+) kg<sup>-1</sup>, respectively.

Table 4.2. Chemical properties of Azolla dry biomass before incorporation

Azolla	Concentration
pH(H <sub>2</sub> O,1:2.5)	7.02
Total nitrogen (%)	5.08
Organic carbon (%)	48
C/N	9.45
Available Phosphorous(mg kg <sup>-1</sup> )	276.40
Cation exchange capacity(cmol(+) kg <sup>-1</sup> )	41.35

### **4.3.3. Effects of Azolla dry biomass and inorganic N on soil chemical properties after harvest of tomato in the selected districts**

#### **4.3.3.1. Soil pH, electrical conductivity, total N and soil organic carbon**

Table 4.3 summarizes the results of soil chemical characteristics after harvest. The combined two-year data (2022/23 and 2023/24) in the both locations demonstrated that the various treatment combinations had a significant influence on the majority of soil chemical parameters. However, the combined use of Azolla dry biomass and inorganic nitrogen fertilizers had no significant influence on soil electrical conductivity at both locations (Table 4.3).

The two-year combined analysis of the Hawassa Zuria location showed that soil pH was significantly affected by the application of Azolla dry biomass with inorganic N. The soil pH decreased by 1.14 units when 15 t ha<sup>-1</sup> of Azolla dry biomass and 138 kg ha<sup>-1</sup> (pH 7.49) were applied, compared to the control treatment with a pH of 8.63 (Table 4.3). This may be due to the fast release of nutrients from the combined application of Azolla dry biomass and inorganic N during the research period into the soil solution. But, in the combined two-year data from the Wondo Genet location, the soil pH and electrical conductivity showed non significant interaction effects of Azolla dry biomass with inorganic N. In both locations, post-harvest results showed that applying Azolladry biomass and inorganic nitrogen fertilizers together considerably enhanced soil chemical characteristics, particularly organic carbon, total nitrogen, available phosphorus, and cation exchange capacity. Combined two-year data (2022/23 and 2023/24) in the Hawassa Zuria and Wondo Genet showed that plots receiving 15 t ha<sup>-1</sup> Azolla dry biomass with 138 kg N ha<sup>-1</sup> had the greatest soil organic carbon levels (4.25% and 5.08%), compared to the control (Table 4.3). This reveals that applying Azolla considerably increases soil organic matter, which is consistent with previous research demonstrating its involvement in nitrogen fixation and organic matter accumulation (Fasusi et al., 2021; Prabakaran et al., 2022). Similarly, Nigussie et al. (2021) reported maximum soil OC under 20 t ha<sup>-1</sup> organic amendments combined with 138 kg N ha<sup>-1</sup> in maize, whereas the lowest levels occurred in the control.

In both locations, the addition of Azolla dry biomass and nitrogen fertilizer significantly improved the total nitrogen content (TN) in the soil (Table 4.3). A two-year combined soil data indicated the highest soil TN of 0.34% for Hawassa Zuria and 0.44% for Wondo Genet soil with the addition of the highest rate of Azolla dry biomass and inorganic N, respectively (Table 4.3).

In both locations, TN increases with increasing application of Azolla dry biomass and inorganic N fertilizers. This implies that the decomposition and mineralization process released additional nitrogen from Azolla. A similar finding was reported by Marzouk et al. (2024) and Setyono and Akbar (2023), who showed that the use of Azolla at 100% (6,800 kg ha<sup>-1</sup>) had a higher TN content. The significant increase in total nitrogen (TN) levels found in the soil, particularly in the Azolla-fertilized treatments, may be attributed to Azolla's nitrogen-fixing capacity. Using nitrogen-fixing cyanobacteria in its symbiotic association (Akhtar et al., 2021), which increases nitrogen availability in the soil as well as decomposition and mineralization of applied Azolla to release additional nitrogen.

At the Hawassa Zuria site, soil organic carbon and total nitrogen levels were lower than those at Wondo Genet. This is may be due to the Wondo Genet soil by nature good fertility status as compared to Hawassa Zuria soils. The observed increases in soil OC across treatments can be attributed to the enrichment of soil organic matter. Total nitrogen, a key indicator of soil fertility and productivity in agroecosystems (Li M. et al., 2022), also responded positively to the treatments. The highest combined application of Azolla dry biomass and nitrogen fertilizer resulted in the greatest improvements in soil OC and TN, while simultaneously reducing soil pH, particularly in Hawassa soils. These findings were consistent with those of Marzoukl et al. (2024), who found that combining Azolla with rice straw inclusion and inorganic NPK in rice paddy improved soil organic carbon content after harvest compared to the control. Similarly, Musse et al. (2020) found that applying liquid slurry (the organic N source) and inorganic N rates resulted in a considerable increase in total nitrogen and soil organic carbon content shortly after green bean harvest.

#### **4.3.3.2. Available phosphorus and cation exchange capacity**

Combined two-year data (2022/23 and 2023/24) in both locations indicate the effect of Azolla dry biomass and inorganic N, as well as their interaction, showed significant effects on the available phosphorus and CEC content of the soil. In Hawassa Zuria and Wondo Genet soil, the highest available phosphorus levels of 17.97 mg kg<sup>-1</sup> and 35.38 mg kg<sup>-1</sup> were respectively recorded with the combined applications of 15 t ha<sup>-1</sup> of Azolla dry biomass and 138 kg N ha<sup>-1</sup>, while the lowest available phosphorus levels were found in control treatments (Table 4.3).

At Hawassa Zuria, available phosphorus levels were lower, while in Wondo Genet they ranged from high to very high, reflecting the influence of basic soil pH in Hawassa and neutral in Wondo Genet. In this study, the combined application of inorganic N fertilizer and Azolla dry biomass significantly enhanced soil available phosphorus. In Hawassa Zuria, the combined application of Azolla dry biomass with inorganic N fertilizer can be help to reduce the soil pH tha led to increase the available P availability. These findings are comparable with those published by Nigussie et al. (2021), who found that combining compost and inorganic N fertilizers considerably increased soil-avaliable phosphorus. In the locations, the combined application of Azolla dry biomass and inorganic nitrogen rates had a significant effect on the CEC of the soil compared to the control (Table 4.3). In both locations, the highest soil CEC content (28.51 cmol(+)/kg and 35.49 cmol(+)/kg) was obtained from the combined application of 15 t ha<sup>-1</sup> of Azolla dry biomass and 138 kg N ha<sup>-1</sup> ofat Hawassa Zuria and Wondo Genet, respectively (Table 4.3). A 15.54% and 20.60% more CEC was attained at Hawassa Zuria and Wondo Genet for the integrated application of 15 t ha<sup>-1</sup> of Azolla dry biomass and 138 kg N ha<sup>-1</sup> as compared to the control (no N applied). This increase in CEC may be attributed to the combined application of Azolla dry biomass and inorganic N on negatively charged colloidal sites, acting as a storehouse for basic cations. These findings align with Tana and Woldesenbet (2017), who found that the use of organic FYM and inorganic fertilizers significantly raised CEC compared to the control. Therefore, the organic carbon/matter in the soil may act as a reservoir for basic cations, ultimately boosting the CEC of the soil. Redda and Kebede (2017) also observed similar results regarding CEC, OM, and OC content of the soil, indicating increases with the integrated use of organic manure and inorganic fertilizers.

Table 4.3 Soil chemical properties after harvest under combined Azolla dry biomass and inorganic N fertilizer treatments over two years (2022/23–2023/24)

Treatment combinations		Hawassa Zuria district						Wondo Genet district					
Azolla (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	pH	EC(dS/m)	OC %	TN	Ava. P(mg kg <sup>-1</sup> )	CEC(cmol(+)/kg)	pH	EC(dS/m)	OC %	TN	Ava. P(mg kg <sup>-1</sup> )	CEC(cmol(+)/kg)
0	0	8.63 <sup>a</sup>	3.12	2.02 <sup>h</sup>	0.17 <sup>f</sup>	8.93 <sup>g</sup>	24.08 <sup>f</sup>	7.23	1.82	3.24 <sup>f</sup>	0.23 <sup>f</sup>	29.44 <sup>l</sup>	28.18 <sup>g</sup>
	46	8.62 <sup>a</sup>	3.09	2.56 <sup>g</sup>	0.17 <sup>f</sup>	9.02 <sup>g</sup>	24.31 <sup>ef</sup>	6.67	2.08	3.71 <sup>e</sup>	0.37 <sup>d</sup>	30.56 <sup>ik</sup>	29.46 <sup>gh</sup>
	92	8.60 <sup>a</sup>	3.26	3.69 <sup>bc</sup>	0.21 <sup>de</sup>	9.22 <sup>fg</sup>	24.40 <sup>ef</sup>	6.98	2.15	4.18 <sub>d</sub>	0.38 <sub>d</sub>	31.60 <sup>ji</sup>	30.32 <sup>gf</sup>
	138	8.60 <sup>a</sup>	2.95	3.10 <sup>de</sup> <sub>f</sub>	0.21 <sup>de</sup>	9.28 <sup>fg</sup>	24.52 <sup>ef</sup>	6.94	1.90	4.26 <sub>d</sub>	0.39 <sup>b</sup> <sub>cd</sub>	31.38 <sup>j</sup>	31.34 <sup>dfe</sup>
5	0	8.50 <sup>b</sup> <sub>ac</sub>	2.88	3.12 <sup>de</sup> <sub>f</sub>	0.19 <sup>ef</sup>	9.41 <sup>fg</sup>	24.87 <sup>ed</sup>	6.71	2.09	4.25 <sub>d</sub>	0.40 <sup>b</sup> <sub>c</sub>	32.08 <sup>gh</sup>	31.07 <sup>fe</sup>
	46	8.53 <sup>b</sup> <sub>a</sub>	3.08	3.25 <sup>de</sup>	0.24 <sup>dc</sup>	9.43 <sup>fg</sup>	24.66 <sup>ef</sup>	6.94	2.04	4.41 <sub>d</sub>	0.40 <sup>b</sup> <sub>c</sub>	32.67 <sup>f</sup>	32.18 <sup>dce</sup>
	92	8.51 <sup>b</sup> <sub>ac</sub>	3.11	2.59 <sup>g</sup>	0.27 <sup>de</sup>	9.40 <sup>fg</sup>	24.40 <sup>ef</sup>	6.69	2.15	4.63 <sup>c</sup> <sub>b</sub>	0.38 <sup>c</sup> <sub>d</sub>	31.98 <sup>hi</sup>	32.57 <sup>dce</sup>
	138	8.54 <sup>a</sup>	3.21	2.73 <sup>fg</sup>	0.30 <sup>ba</sup>	10.97 <sup>fe</sup>	24.58 <sup>ef</sup>	6.78	2.19	4.54 <sup>c</sup>	0.40 <sup>b</sup> <sub>c</sub>	32.08 <sup>gh</sup>	32.66 <sup>dc</sup>
10	0	8.32 <sup>d</sup>	3.09	3.35 <sup>cd</sup>	0.29 <sup>b</sup>	11.47 <sup>e</sup> <sub>d</sub>	25.46 <sup>cd</sup>	6.67	2.29	4.72 <sub>b</sub>	0.38 <sup>c</sup> <sub>d</sub>	32.25 <sup>fg</sup>	33.40 <sup>bc</sup>

	46	8.36 <sup>d</sup> <sub>c</sub>	2.90	3.17 <sup>de</sup>	0.29 <sup>b</sup>	12.17 <sup>c</sup> <sub>ed</sub>	25.81 <sup>cb</sup>	6.67	1.93	4.65 <sup>c</sup> <sub>b</sub>	0.39 <sup>b</sup> <sub>cd</sub>	32.62 <sup>f</sup>	33.25 <sup>c</sup>	
	92	8.38 <sup>b</sup> <sub>dc</sub>	2.93	3.63 <sup>bc</sup>	0.31 <sup>ba</sup>	11.80 <sup>e</sup> <sub>d</sub>	25.87 <sup>cb</sup>	7.06	2.08	4.72 <sub>b</sub>	0.41 <sup>b</sup>	33.25 <sup>de</sup>	32.02 <sup>dce</sup>	
	13 8	7.37 <sup>d</sup> <sub>c</sub>	3.09	3.64 <sup>bc</sup>	0.22 <sup>de</sup>	12.96 <sup>c</sup> <sub>bd</sub>	24.99 <sup>ed</sup>	6.62	1.91	4.53 <sup>c</sup>	0.40 <sup>b</sup> <sub>c</sub>	33.33 <sup>d</sup>	32.47 <sup>dce</sup>	
	15	0	7.55 <sup>e</sup>	2.90	3.49 <sup>bc</sup>	0.22 <sup>de</sup>	12.91 <sup>c</sup> <sub>bd</sub>	25.82 <sup>cb</sup>	6.73	1.80	4.57 <sup>c</sup> <sub>b</sub>	0.42 <sup>b</sup> <sub>a</sub>	33.95 <sup>b</sup>	32.99 <sup>c</sup>
	46	7.56 <sup>e</sup>	2.81	3.56 <sup>bc</sup>	0.32 <sup>ba</sup>	13.90 <sup>c</sup> <sub>b</sub>	28.11 <sup>a</sup>	7.04	1.88	4.72 <sub>b</sub>	0.39 <sup>b</sup> <sub>cd</sub>	33.84 <sup>bc</sup>	32.42 <sup>dce</sup>	
	92	7.54 <sup>e</sup>	2.91	3.85 <sup>ba</sup>	0.32 <sup>ba</sup>	14.13 <sup>b</sup>	26.39 <sup>b</sup>	6.69	2.04	5.06 <sup>a</sup>	0.42 <sup>b</sup> <sub>a</sub>	33.49 <sup>cd</sup>	34.96 <sup>ba</sup>	
	13 8	7.49 <sup>e</sup>	2.88	4.25 <sup>a</sup>	0.34 <sup>a</sup>	17.97 <sup>a</sup>	28.51 <sup>a</sup>	6.61	1.93	5.08 <sup>a</sup>	0.44 <sup>a</sup>	35.38 <sup>a</sup>	35.49 <sup>a</sup>	
<b>LSD</b> <b>(5%)</b>		<b>0.16</b>	<b>ns</b>	<b>0.43</b>	<b>0.046</b>	<b>1.86</b>	<b>0.69</b>	<b>ns</b>	<b>ns</b>	<b>0.17</b>	<b>0.03</b>	<b>0.45</b>	<b>1.57</b>	
<b>CV</b> <b>(%)</b>		<b>11.60</b>	<b>9.15</b>	<b>15.56</b>	<b>10.75</b>	<b>9.83</b>	<b>16.45</b>	<b>9.02</b>	<b>16.20</b>	<b>8.28</b>	<b>10.75</b>	<b>5.12</b>	<b>10.93</b>	

EC: electrical conductivity; OC: organic carbon; TN: total nitrogen; Ava.P: available phosphorus; CEC: cation exchange capacity. Means followed by the same letter (s) within the column are not significantly different at  $P \leq 0.05$ .

#### **4.3.4. Effect of Azolla dry biomass and inorganic N fertilizers on yield component parameters on tomato in the study areas**

##### **4.3.4.1. Plant height (cm)**

The combined two-year data (2022/23 and 2023/24) in both locations showed a significant interaction between Azolla dry biomass and inorganic nitrogen fertilizers on tomato plant height ( $P < 0.05$ ) (Table 4.4). The application of 15 t ha<sup>-1</sup> Azolla dry biomass with 138 kg N ha<sup>-1</sup> resulted in the tallest plants, measuring 69.21 cm and 76.23 cm in Hawassa Zuria and Wondo Genet, respectively (Table 4.4). As reported by Razavipour et al. (2018), these findings indicate that Azolla promotes tomato development by increasing soil nitrogen availability and organic matter content, hence improving nutrient uptake and plant growth.

The observed increase in plant height caused by the application of Azolla dry biomass and inorganic N fertilizers as soil amendments could be related to improvements in the soil's physical, chemical, and biological qualities. This ensures that plants receive an adequate supply of nutrients, allowing for optimal vegetative growth. Dry Azolla ability to give diverse plant nutrients while also creating a favorable growing environment by improving soil moisture and nutritional status may contribute to plant development and performance overall. In agreement with this, Thomas et al. (2019) stated that organic fertilizers can help regulate nutrient levels in the soil, which may fluctuate with mineral fertilization. Given that nitrogen is a crucial component of protein and a fundamental building material for cells, as well as a key element in enzymes, the metabolic processes of plants greatly benefit from the addition of nitrogen fertilizer. The results of this experiment align with those of Zeleke et al. (2024), who reported that organic manure and inorganic fertilizer contribute to the essential nutrient supply during the growth stage, leading to increased tomato plant height. Similarly, Paszt et al. (2025) observed a significant increase in plant height in crop with the application of Azolla compared to the control treatment.

##### **4.3.4.2. Number of fruits per plant**

Regarding the number of fruits per plant, all treatments produced significantly higher values than the control. Combined two-year data in the both locations indicated that the interaction of Azolla dry biomass with inorganic N generally enhanced fruit number relative to the control (Table 4.4). The highest number of fruit per plant was recorded at Hawassa Zuria and Wondo Genet with 32.34 and 40.17 fruits per plant, respectively, under 15 t ha<sup>-1</sup> Azolla dry biomass combined with

138 kg N ha<sup>-1</sup> (Table 4.4). Compared to Hawassa Zuria (Cambisols), the Wondo Genet site (Andosols) produced a greater number of fruits per plant, likely due to its higher organic matter content, elevated total nitrogen levels, and superior cation exchange capacity (CEC), which together reflect more favorable soil fertility conditions. Similar findings were reported by Jembere et al. (2024), who demonstrated that Andosols possess higher organic matter, CEC, and total nitrogen than Cambisols, thereby enhancing soil fertility and contributing to increased fruit production per plant. In both locations, the number of fruits per plant increased with the combined application of Azolla dry biomass and inorganic N, regardless of application rate. Treatments such as Azolla 0 t ha<sup>-1</sup> + 46 kg N ha<sup>-1</sup> and Azolla dry biomass 5 t ha<sup>-1</sup> + 0 N kg ha<sup>-1</sup> produced significantly fewer fruits compared to other treatment combinations (Table 4.4). Across the two locations, integrating Azolla dry biomass with inorganic N significantly enhanced number of fruit per plant relative to the control. The increase in fruit number is likely attributable to improved nutrient availability through the mineral fertilizer and mineralization of organic inputs, preventing nutrient stress during critical growth stages and maximizing fruit production. These findings are consistent with previous reports indicating that combined organic and inorganic nutrient management enhances tomato yield and fruit number (Gebretsadkan and Assefa, 2015; Biswas et al., 2015). Nitrogen, in particular, plays a key role in increasing both fruit number and fruit size (Adekiya et al., 2017).

#### **4.3.4.3. Number of clusters per plant and number of fruits per cluster**

The combined data from two years in the both locations revealed significant differences in the interaction effects of Azolla dry biomass and inorganic nitrogen on the number of clusters per plant and the number of fruits per cluster (Table 4.4). At the Hawassa Zuria and Wondo Genet locations, the treatment with combined applications of 15 t ha<sup>-1</sup> of Azolla dry biomass with 138 kg N ha<sup>-1</sup> resulted in a greater number of clusters per plant (18.34 and 25.67) and a higher number of fruits per cluster (8.34 and 10.34) compared to the control group, respectively (Table 4.4). The control group recorded the lowest number of clusters per plant and the fewest fruits per cluster. This may be attributed to the synergistic effect of Azolla and inorganic nitrogen fertilizers, which enhances nutrient uptake and assimilation, thereby increasing the availability of native soil nutrients through elevated biological activity.

Applying Azolla dry and inorganic nitrogen fertilizers at rates of 15 t ha<sup>-1</sup> and 138 kg N ha<sup>-1</sup>, respectively, resulted in an increase of approximately 55% in the number of fruit clusters and 50.6% in the number of fruits per plant compared to the control treatment. Zeleke et al. (2024) also reported similar findings in a pot experiment, where the combination of 0.69 g of inorganic nitrogen (equivalent to 138 kg ha<sup>-1</sup>) and 75 g of Azolla (equivalent to 15 t ha<sup>-1</sup>) led to an increase in the number of fruits. According to Ogundare et al. (2015), the number of fruits per plant, fruit yield per plot, and overall tomato yield are significantly influenced by the combined application of organic and inorganic fertilizers. Similar findings were reported by Benti et al. (2021), who indicated that the integration of organic with inorganic nitrogen fertilizers led to increased tomato yields.

#### **4.3.4.5. Average fruit weight**

The combined two-year data analysis results in both locations indicated that the average fruit weight of tomato was significantly ( $P < 0.05$ ) affected by the interaction of Azolla dry biomass with inorganic N fertilizer (Table 4.4). The application of 15 t ha<sup>-1</sup> of Azolla with 138 kg N ha<sup>-1</sup> resulted in the maximum average fruit weight of tomatoes in both locations (78.03 g in Hawassa Zuria and 95.43 g in Wondo Genet), while the minimum average fruit weight (39.91 g in Hawassa Zuria and 38.22 g in Wondo Genet) was observed in the control plot (Table 4.4). This difference might be attributed to the nitrogen (N) released from Azolla and inorganic N into the soil solution, promoting better plant growth and development. Additionally, the addition of macro- and micronutrients from dry Azolla biomass (DA) could have improved soil pH, organic carbon, total nitrogen, phosphorus, and cation exchange capacity. Nitrogen plays a crucial role in increasing fruit production and size, as indicated by Adekiya et al. (2017).

The increase in tomato fruit weight in N-treated groups may be due to tomatoes being a component of chlorophyll, which supports cell division and elongation during plant growth, resulting in better fruit production. This study aligns with the findings of Benti et al. (2021), who observed a significant impact on the average fruit weight of tomato plants with the combined application of organic fertilizer and inorganic N fertilizer. Similar improvements in growth parameters and tomato fruit weight were reported in Bangladesh by Islam et al. (2017), while enhanced performance of integrated nutrient management practices was noted in vegetables like cabbage and mustard by Ullah et al. (2008).

Table 4.4. Effects of combined Azolla dry biomass and inorganic N fertilizer application on tomato yield components during the 2022/23–2023/24 cropping seasons

Treatment combinations		Hawassa Zuria district					Wondo Genet district				
Azolla (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	PH(cm)	NCP	NFrPC	FW(g)	NFP	PH(cm)	NCP	NFrPC	FW(g)	NFP
0	0	29.45 <sup>i</sup>	8.67 <sup>h</sup>	2.00 <sup>h</sup>	38.91 <sup>k</sup>	9.67 <sup>h</sup>	38.82 <sup>p</sup>	8.84 <sup>egf</sup>	3.67 <sup>j-m</sup>	38.22 <sup>n</sup>	12.49 <sup>mno</sup>
	46	42.74 <sup>h</sup>	10.00 <sup>gh</sup>	3.00 <sup>gh</sup>	47.31 <sup>fg</sup>	11.00 <sup>h</sup>	46.151 <sup>mn</sup>	12.34 <sup>c-g</sup>	4.17 <sup>g-l</sup>	47.09 <sup>m</sup>	14.84 <sup>klm</sup>
	92	45.78 <sup>gh</sup>	13.00 <sup>de</sup>	4.00 <sup>fg</sup>	47.19 <sup>fg</sup>	12.34 <sup>gh</sup>	50.06 <sup>jkl</sup>	12.50 <sup>c-g</sup>	4.50 <sup>g-i</sup>	49.77 <sup>klm</sup>	17.67 <sup>kl</sup>
	138	46.39 <sup>efg</sup>	12.00 <sup>de</sup>	6.00 <sup>cd</sup>	48.42 <sup>fg</sup>	14.34 <sup>g</sup>	51.93 <sup>h-k</sup>	13.33 <sup>c-g</sup>	5.17 <sup>e-k</sup>	52.56 <sup>jk</sup>	20.83 <sup>j</sup>
5	0	46.67 <sup>egh</sup>	11.00 <sup>fg</sup>	3.66 <sup>fg</sup>	46.19 <sup>g</sup>	12.00 <sup>gh</sup>	47.21 <sup>lmn</sup>	12.66 <sup>c-f</sup>	3.83 <sup>i-m</sup>	46.82 <sup>m</sup>	15.17 <sup>klm</sup>
	46	50.48 <sup>egh</sup>	13.00 <sup>de</sup>	5.34 <sup>de</sup>	51.28 <sup>ef</sup>	21.34 <sup>e</sup>	54.45 <sup>fgh</sup>	13.17 <sup>c-f</sup>	5.00 <sup>g-l</sup>	53.72 <sup>ij</sup>	23.49 <sup>g-j</sup>
	92	52.50 <sup>de</sup>	14.00 <sup>bcd</sup>	6.00 <sup>cd</sup>	56.77 <sup>d</sup>	22.67 <sup>e</sup>	55.50 <sup>fgh</sup>	14.00 <sup>cde</sup>	6.00 <sup>c-f</sup>	60.42 <sup>gh</sup>	25.17 <sup>d-h</sup>
	138	59.69 <sup>bc</sup>	13.67 <sup>ab</sup>	6.67 <sup>bc</sup>	62.17 <sup>c</sup>	23.34 <sup>de</sup>	53.67 <sup>g-j</sup>	14.99 <sup>b-e</sup>	6.67 <sup>b-e</sup>	62.87 <sup>efg</sup>	25.66 <sup>d-g</sup>
10	0	49.08 <sup>efg</sup>	11.00 <sup>fg</sup>	4.00 <sup>fg</sup>	47.92 <sup>fg</sup>	17.67 <sup>f</sup>	52.58 <sup>h-k</sup>	11.83 <sup>c-g</sup>	4.83 <sup>f-k</sup>	47.00 <sup>m</sup>	20.84 <sup>j</sup>
	46	49.60 <sup>efg</sup>	13.67 <sup>cde</sup>	5.34 <sup>de</sup>	54.68 <sup>de</sup>	22.00 <sup>e</sup>	55.95 <sup>e-h</sup>	13.17 <sup>c-f</sup>	5.50 <sup>c-f</sup>	53.78 <sup>ijk</sup>	24.16 <sup>e-i</sup>
	92	55.12 <sup>de</sup>	13.67 <sup>cde</sup>	6.00 <sup>cd</sup>	67.08 <sup>b</sup>	26.00 <sup>bc</sup>	61.00 <sup>cd</sup>	15.83 <sup>b-e</sup>	6.00 <sup>c-f</sup>	66.46 <sup>de</sup>	28.33 <sup>c</sup>
	138	60.25 <sup>bc</sup>	14.34 <sup>bcd</sup>	7.34 <sup>ab</sup>	69.29 <sup>b</sup>	26.34 <sup>bc</sup>	71.67 <sup>b</sup>	16.50 <sup>bcd</sup>	6.83 <sup>b-e</sup>	71.22 <sup>c</sup>	33.67 <sup>b</sup>
15	0	59.79 <sup>bc</sup>	13.67 <sup>cde</sup>	4.34 <sup>ef</sup>	46.28 <sup>g</sup>	23.34 <sup>de</sup>	68.47 <sup>b</sup>	13.50 <sup>c-f</sup>	5.66 <sup>d-h</sup>	46.54 <sup>m</sup>	23.50 <sup>g-j</sup>
	46	60.38 <sup>b</sup>	16.67 <sup>ab</sup>	6.00 <sup>cd</sup>	61.83 <sup>c</sup>	24.00 <sup>cde</sup>	70.67 <sup>b</sup>	18.83 <sup>abc</sup>	6.64 <sup>c-f</sup>	63.32 <sup>efg</sup>	26.84 <sup>cde</sup>
	92	63.38 <sup>b</sup>	16.34 <sup>ab</sup>	7.34 <sup>ab</sup>	67.90 <sup>b</sup>	27.67 <sup>b</sup>	70.67 <sup>b</sup>	21.42 <sup>ab</sup>	7.84 <sup>bc</sup>	70.94 <sup>c</sup>	33.00 <sup>b</sup>

	138	69.21 <sup>a</sup>	18.34 <sup>a</sup>	8.34 <sup>a</sup>	78.03 <sup>a</sup>	32.34 <sup>a</sup>	76.23 <sup>a</sup>	25.67 <sup>a</sup>	10.17 <sup>a</sup>	95.43 <sup>a</sup>	40.17 <sup>a</sup>
<b>Mean</b>		<b>51.43</b>	<b>13.32</b>	<b>5.32</b>	<b>55.70</b>	<b>20.38</b>	<b>58.59</b>	<b>14.88</b>	<b>5.78</b>	<b>57.88</b>	<b>24.11</b>
<b>LSD (5%)</b>		<b>4.55</b>	<b>2.94</b>	<b>1.05</b>	<b>4.63</b>	<b>2.97</b>	<b>4.25</b>	<b>5.02</b>	<b>1.76</b>	<b>4.09</b>	<b>1.84</b>
<b>CV (%)</b>		<b>5.23</b>	<b>13.51</b>	<b>11.84</b>	<b>4.99</b>	<b>8.73</b>	<b>7.59</b>	<b>10.03</b>	<b>14.77</b>	<b>13.45</b>	<b>5.93</b>

Means followed by the same letter(s) within a column are not significantly different at  $p \leq 0.05$ . PH= plant height, NCPP= number of cluster per plant, NFPP= number of fruits per plant, NFrPC= number of fruit per cluster, FW (g) = fruit weight.

#### **4.3.4.6. Number of leaves per plant**

The combined two-year data in the both locations indicated that the interaction of Azolla dry biomass with inorganic N fertilizers had a significant effect on the number of leaves per plant ( $P \leq 0.01$ ) (Table 4.5). In both locations, treatments combining Azolla biomass with inorganic N differed significantly from the control and generally produced higher yields and improved yield components (Table 4.5). The analyzed data revealed significant effects of Azolla dry biomass and inorganic nitrogen fertilizer at both locations, with a significance level of  $P \leq 0.05$ . In Hawassa Zuria and Wondo Genet, the locations show the average maximum number of leaves per plant observed was 41.00 and 46.00, respectively, under treatment group ( $15 \text{ t ha}^{-1}$  of Azolla dry biomass +  $138 \text{ kg N ha}^{-1}$ ). Conversely, in both locations, the control treatment gave the minimum average number of leaves per plant. The increase in the number of leaves per plant may be attributed to greater nitrogen uptake, ultimately enhancing vegetative growth and the number of leaves per plant. These results indicate that the growth of the tomatoes increased, ensuring a greater number of branches than in the control group. Nitrogen fertilization increases the number of leaves per plant (Biwas et al., 2015). These findings agree with those of Zeleke et al. (2024), who suggested that the combined application of Azolla and inorganic N fertilizer at  $0.69 \text{ g pot}^{-1}$  ( $138 \text{ kg N ha}^{-1}$ ) +  $75 \text{ g pot}^{-1}$  ( $15 \text{ t ha}^{-1}$  of Azolla) increased the number of leaves per plant through vegetative growth. According to Biswas et al. (2015), tomato plants treated with  $150 \text{ kg}$  of nitrogen per hectare had the greatest number of clusters, which is consistent with the findings of the present study. Fiseha (2014) also observed that tomatoes tend to form more clusters as the amount of nitrogen increases. These results confirmed the findings of Reddy et al. (2022) that the combined application of organic and inorganic results in an increase in plant height, number of leaves per plant, and number of fruits per plant.

#### **4.3.4.7. Number of branches per plant**

The combined two-year data (2022/23 and 2023/24) in the both locations showed that the interaction of Azolla dry biomass with inorganic N significantly enhanced the number of branches per plant. Analysis indicated a highly significant effect ( $P \leq 0.01$ ) among treatments (Table 4.5), consistent with Sharma and Rastogi (1993), who reported significant differences in branch number between treated and control plants.

At the Hawassa Zuria and Wondo Genet sites, plants treated with 15 t ha<sup>-1</sup> Azolla dry biomass and 138 kg N ha<sup>-1</sup> fertilizer produced the most branches per plant (5.50 and 6.84 tomatoes, respectively). Notably, the control had the lowest branches per plant (2.34 and 2.67 of tomato, respectively) in the Hawassa Zuria and Wondo Genet locations (Table 4.5). Due to their synergistic effects on plant development and nutrient availability, the combined application of Azolla and inorganic nitrogen (N) fertilizers in tomato growing may result in more branches. While inorganic fertilizers supply easily accessible nitrogen for quick development, which leads to more branching, organic fertilizers enhance soil structure and microbial activity. In general, the increased number of branches per plant could be attributed to a balanced nutrient supply in the root zone, which may have improved the plant's nutrient uptake for better growth. The superiority observed due to the combined application of organic and inorganic nutrient sources compared to the control (without fertilizer) may be due to the direct promotion of root growth (Glala et al., 2010) and the release of fixed nutrients, thereby increasing the concentration and availability of nutrients in the root zone and tomato plant growth and development (Biramo, 2017). These findings were also consistent with those of Abdalla et al. (2001), Glala et al. (2010), and Glala et al. (2012), who reported similar results in pepper plants.

#### **4.3.5. Effect of Azolla dry biomass and inorganic nitrogen on yield of tomato**

##### **4.3.5.1. Interaction effects of Azolla dry biomass and inorganic N fertilizers on marketable and total fruit yield**

The combined two-year data in Hawassa Zuria and Wondo Genet indicated that the interaction of Azolla dry biomass and inorganic N fertilizers significantly influenced both marketable and total tomato fruit yields ( $P < 0.01$ ) (Figures 4.1a and 4.1b and Table 4.5). The highest yields were recorded with the integrated application of 15 t ha<sup>-1</sup> Azolla dry biomass and 138 kg N ha<sup>-1</sup>, producing marketable yields of 23.76 t ha<sup>-1</sup> and 26.35 t ha<sup>-1</sup> (Figures 4.1 a and b) and total yields of 26.17 t ha<sup>-1</sup> and 29.03 t ha<sup>-1</sup> in Hawassa Zuria and Wondo Genet, respectively (Table 4.5). In contrast, the control treatments resulted in the lowest marketable (4.11 t ha<sup>-1</sup> and 5.42 t ha<sup>-1</sup>) and total yields (4.94 t ha<sup>-1</sup> and 6.22 t ha<sup>-1</sup>). The combined application increased marketable fruit yields by 80.37% and 78.57% and total fruit yields by 54.60–80.37% and 51.33–78.57% in Hawassa Zuria and Wondo Genet, respectively (Table 4.5). However, their interaction consistently enhanced marketable and total yields across both locations. The observed yield

improvements are attributed to the positive effects of Azolla dry biomass and inorganic N on vegetative growth, leaf area, photosynthetic capacity, and assimilate partitioning towards fruit development. Variations in yield response between locations likely reflect differences in soil characteristics and fertility status.

Similar findings have been reported by several studies. Marzouketal et al. (2024) showed that Azolla combined with rice straw improved rice yield and nitrogen use efficiency, while Widiastuti (2017) observed increased vegetable yields with Azolla application. These results suggest that integrating Azolla with inorganic N enhances vegetative growth, photosynthetic capacity, and assimilate partitioning, leading to more fruits, better fruit quality, and higher marketable yields. Nnakiwala (2021) also reported increased marketable yields with Azolla, consistent with earlier studies by Singh (1977) and Mahmud et al. (2016), who found that Azolla incorporation boosted rice grain and straw yield. Azolla has further been shown to improve soil fertility, microbial populations, and nutrient cycling (Kollah et al., 2016; Guo et al., 2019; Zhang et al., 2020). Similarly, Adhikary et al. (2016) reported the highest tomato yields with 50% organic and inorganic fertilizer integration, highlighting Azolla's role as a sustainable nitrogen source.

Bipradas et al. (2016) reported that the combined application of 50% organic fertilizer and inorganic fertilizer led to the highest tomato production. Similarly, Saha et al. (2017) noted that the maximum tomato yield was achieved when applying a combination of organic fertilizer and 50% of the recommended inorganic fertilizer dose compared to using only organic or inorganic fertilizer. In contrast, some authors have reported that tomato plants fertilized with inorganic N fertilizers produce relatively high fruit yields due to the presence of soluble inorganic nitrogen and other nutrients, resulting in relatively high yields (Chassy et al., 2006; Riahi et al., 2009). The results of this study were consistent with those of Beyene and Mulu (2019), who reported that the yield of tomatoes increased as the rate of nitrogen fertilizer increased. Also, this finding is consistent with the findings of Zeleke et al. (2024), who showed that the combined application of 0.69 g N pot<sup>-1</sup> and 75 g Azolla dry biomass pot<sup>-1</sup> resulted in the highest marketable quantity of tomatoes. Conversely, the lowest yield was observed in the control treatment (Zeleke et al., 2024). The increase in yield component parameters due to the application of organic and inorganic fertilizers may be attributed to the improved development of the root system and the potential synthesis of plant growth hormones. These results confirmed the earlier results of Singh

et al. (2018), who reported that the incorporation of Azolla biomass into the soil always increased the grain and straw yield of rice.

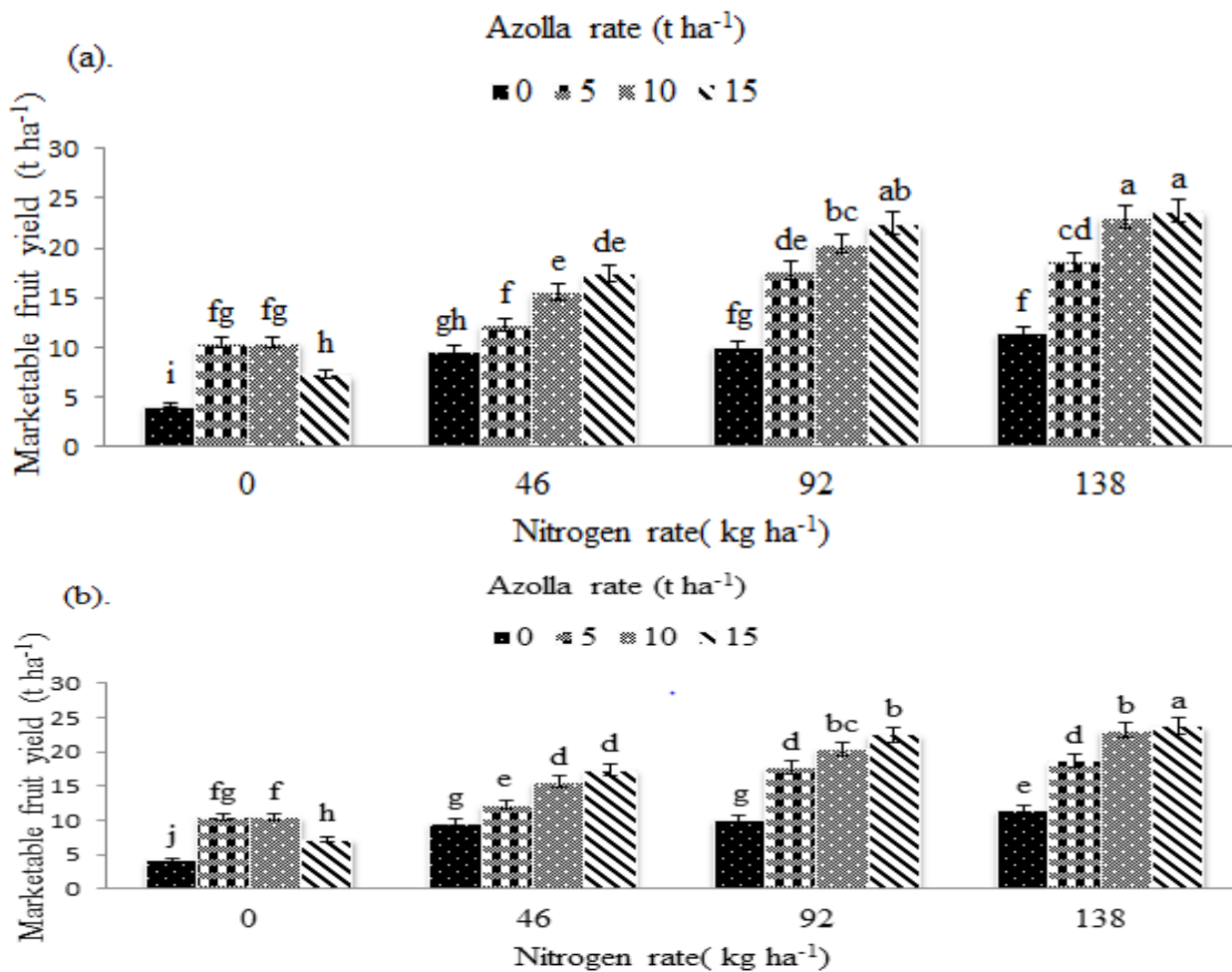


Figure 4.1. Interaction effects of Azolla dry biomass and inorganic N on marketable fruit yield of tomato at (a) Hawassa Zuria and (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

Table 4.5. The interaction effects of Azolla dry biomass and nitrogen on total yield and yield component parameters of tomato in both districts

Treatment combinations		Hawassa Zuria district			Wondo Genet district		
Azolla (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	NLPP	NB	Total yield(t ha <sup>-1</sup> )	NLPP	NB	Total yield(t ha <sup>-1</sup> )
0	0	24.00 <sup>k</sup>	2.67 <sup>ef</sup>	4.98 <sup>i</sup>	25.00 <sup>mn</sup>	2.88 <sup>hi</sup>	6.22 <sup>op</sup>
	46	22.00 <sup>l</sup>	2.33 <sup>f</sup>	10.4 <sup>gh</sup>	25.50 <sup>mn</sup>	3.50 <sup>g</sup>	12.78 <sup>jkl</sup>
	92	26.67 <sup>i</sup>	4.00 <sup>bc</sup>	12.14 <sup>fg</sup>	27.67 <sup>lm</sup>	2.88 <sup>hi</sup>	12.96 <sup>jkl</sup>
	138	34.34 <sup>e</sup>	3.67 <sup>cd</sup>	13.80 <sup>f</sup>	32.34 <sup>ghi</sup>	4.34 <sup>de</sup>	15.86 <sup>ghi</sup>
5	0	30.34 <sup>g</sup>	2.33 <sup>f</sup>	11.65 <sup>fg</sup>	26.84 <sup>lmn</sup>	2.88 <sup>hi</sup>	12.81 <sup>jkl</sup>
	46	30.66 <sup>g</sup>	3.66 <sup>d</sup>	13.59 <sup>f</sup>	28.84 <sup>jkl</sup>	3.88 <sup>ef</sup>	14.98 <sup>ijk</sup>
	92	28.00 <sup>h</sup>	4.34 <sup>bc</sup>	19.82 <sup>d</sup>	34.83 <sup>efg</sup>	4.50 <sup>cd</sup>	19.83 <sup>efg</sup>
	138	32.33 <sup>f</sup>	4.33 <sup>bc</sup>	21.47 <sup>cd</sup>	34.84 <sup>efg</sup>	4.17 <sup>ef</sup>	21.85 <sup>def</sup>
10	0	27.00 <sup>h</sup>	3.34 <sup>de</sup>	11.77 <sup>fg</sup>	28.84 <sup>jkl</sup>	3.50 <sup>g</sup>	15.83 <sup>ghi</sup>
	46	26.34 <sup>i</sup>	3.67 <sup>cd</sup>	17.32 <sup>e</sup>	26.84 <sup>lm</sup>	3.88 <sup>ef</sup>	18.65 <sup>fgh</sup>
	92	40.66 <sup>bc</sup>	4.66 <sup>bc</sup>	22.85 <sup>bc</sup>	39.67 <sup>bc</sup>	4.17 <sup>ef</sup>	25.74 <sup>abc</sup>
	138	41.34 <sup>b</sup>	5.00 <sup>b</sup>	26.04 <sup>a</sup>	41.34 <sup>b</sup>	5.50 <sup>b</sup>	28.33 <sup>ab</sup>
15	0	32.34 <sup>f</sup>	4.34 <sup>bc</sup>	8.34 <sup>h</sup>	33.84 <sup>fgh</sup>	4.50 <sup>cd</sup>	9.72 <sup>mn</sup>
	46	37.34 <sup>d</sup>	4.67 <sup>bc</sup>	19.83 <sup>d</sup>	36.00 <sup>e</sup>	4.34 <sup>de</sup>	22.19 <sup>de</sup>

	92	41.34 <sup>b</sup>	5.00 <sup>b</sup>	24.46 <sup>ab</sup>	41.84 <sup>b</sup>	5.50 <sup>b</sup>	27.85 <sup>ab</sup>
	138	44.62 <sup>a</sup>	6.67 <sup>a</sup>	26.17 <sup>a</sup>	46.00 <sup>a</sup>	6.84 <sup>a</sup>	29.03 <sup>a</sup>
<b>Mean</b>		<b>32.46</b>	<b>3.98</b>	<b>16.54</b>	<b>33.14</b>	<b>4.20</b>	<b>18.41</b>
<b>LSD (5%)</b>		<b>1.53</b>	<b>ns</b>	<b>2.44</b>	<b>2.75</b>	<b>0.36</b>	<b>3.34</b>
<b>CV (%)</b>		<b>5.77</b>	<b>17.31</b>	<b>8.87</b>	<b>6.14</b>	<b>15.86</b>	<b>8.84</b>

Means followed by the same letter(s) within a column are not significantly different at  $p \leq 0.05$ . NLPP=number of leaves per plant; NB= number of branches per plant; and TFY= total fruit yield.

### **4.3.6 Effect of Azolla dry biomass and inorganic nitrogen fertilizers on N concentration and nitrogen uptake in tomato plant**

#### **4.3.6.1. Nitrogen concentration in shoot and fruit of tomato plant**

The combined two-year data (2022/23 and 2023/24) in Hawassa Zuria and Wondo Genet showed that the interaction of Azolla dry biomass and inorganic N fertilizers significantly influenced nitrogen concentrations in tomato shoots and fruits ( $P \leq 0.05$ ; Figures 4.2a and b). Treatments with Azolla dry biomass and inorganic N consistently outperformed the control, with the combination of  $15 \text{ t ha}^{-1}$  Azolla dry biomass +  $138 \text{ kg N ha}^{-1}$  recording the highest fruit nitrogen contents (4.18% and 4.24%) and shoot nitrogen contents (4.98% and 5.14%). The increase in shoot nitrogen concentration with higher N rates may be attributed to enhanced availability of N, healthier root systems, and denser canopies, resulting in greater biological accumulation (Nigussie et al., 2021). Across both locations, the lowest values of nitrogen uptake were observed in the control treatments. This aligns with findings from Paul (2007), who noted that higher nitrogen applications accelerated nitrogen uptake by tomatoes, resulting in higher nitrogen content in tomato shoots.

Furthermore, the increase in fruit nitrogen concentration at higher nitrogen rates may be due to the sufficient availability of nitrogen, which enhances nitrogen mobilization to the fruit during the fruit development stage. Similar results were reported by Arduini et al. (2005) and Wang et al. (2024) in wheat and tomato crops, respectively. Interestingly, Jalpha et al. (2024) found that higher fruit nitrogen and shoot nitrogen concentrations were associated with lower fruit yield, while lower fruit nitrogen concentrations were linked to higher fruit yield in the fall.

The majority of nitrogen accumulation in the whole plant comes from the shoots, indicating that most nitrogen is allocated to vegetative growth rather than reproductive growth. The nutrient removal from harvested crops depends on factors such as yield, nutrient concentration, variety, soil, and environmental conditions. The positive effect of Azolla on nutrient uptake may be linked to its role in improving soil fertility and nutrient availability. Zadeh (2014) reported maximum N uptake in rice grain and straw with  $5 \text{ t ha}^{-1}$  Azolla, while Setiawati et al. (2018) found that Azolla compost improved available N and P in soil, enhancing nutrient content in paddy plants.

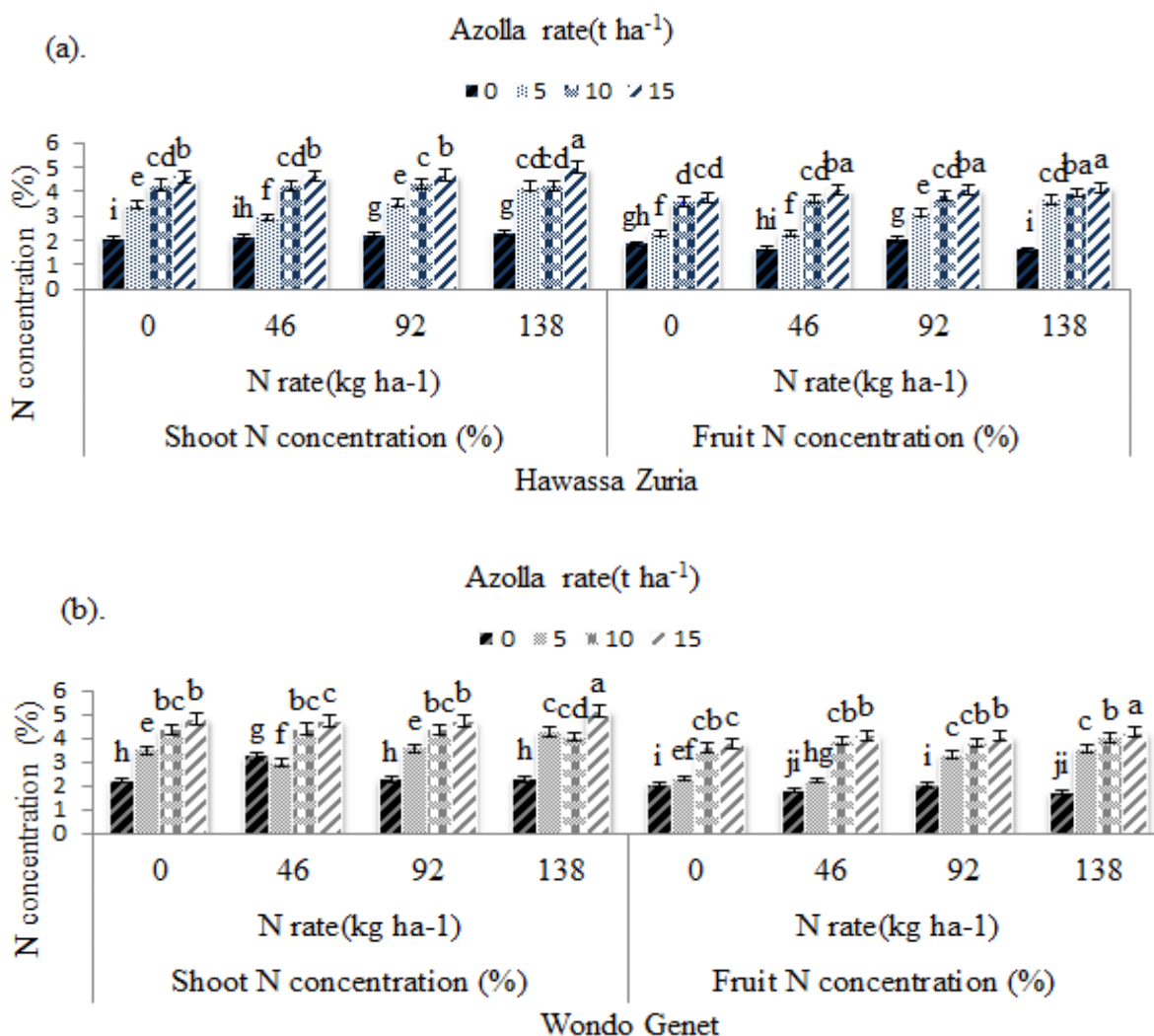


Figure 4.2. Interaction effect of Azolla dry biomass and N fertilizers on shoot and fruit N concentration of tomato plant (a) Hawassa Zuria and (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

#### 4.3.6.2. Nitrogen uptake in shoot and fruit of tomato plant

Combined analysis of two years data (2022/23 and 2023/24) in both locations, the interaction between Azolla dry biomass and inorganic N fertilizers had a significant ( $P \leq 0.05$ ) effects on shoot, fruit and total N uptake of tomato plant (Figures 4.3a and b). The maximum N uptake by tomato shoots and fruit was reported in plots treated with a combined application of Azolla dry biomass as a source of organic nitrogen, or with inorganic N coupled with either half Azolla or inorganic nitrogen (Figures 4.3 a and b). These treatments increased uptake by more than double (100%) as compared to the control. In Hawassa Zuria location, the highest shoot, fruit and total nitrogen uptake were  $1468.24 \text{ kg ha}^{-1}$ ,  $1093.48 \text{ kg ha}^{-1}$  and  $2561.72 \text{ kg ha}^{-1}$ , respectively these values were obtained from the treatment combinations of Azolla dry biomass  $15 \text{ t ha}^{-1}$  with 138

kg N ha<sup>-1</sup> compared to the control (Figure 4.3a and b). In Wondo Genet location, the highest shoot, fruit, and total nitrogen uptake were 1769.18 kg ha<sup>-1</sup>, 1486.94 kg ha<sup>-1</sup>, and 3256.12 kg ha<sup>-1</sup>, respectively and the values were also obtained from the treatment combinations of Azolla dry biomass 15 t ha<sup>-1</sup> with 138 kg ha<sup>-1</sup> compared to the control (Figure 4.3a and b). In the across locations of this study results show that higher nitrogen application levels increase the uptake of shoot, fruit, and total tomato plant nitrogen. This could be due to the fact that applying a large amount of N improves N uptake by increasing N availability (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) in the soil. This immediately meets the plant's need for this essential nutrient. Nitrogen availability stimulates plant growth, root development, photosynthetic capacity, and overall vigor. This allows the plant to absorb more nutrients (Lemma et al., 2023). Tomato total N uptake showed an increasing trend with increase in combined application of Azolla with inorganic N fertilizers. Tomato shoots, fruit, and total nitrogen uptake increased significantly after being treated with Azolla and inorganic nitrogen (Figure 4.3a and b). This result indicates that higher available nutrients, as shown in (Figure 4.3a and b), had a positive impact on the increase in total nitrogen uptake by the plants. The availability of nitrogen in the soil determines the amount that can be absorbed by tomato plants. The total nitrogen uptake of the tomato plants in this study increased after the application of Azolla and inorganic nitrogen fertilizers.

Also similar findings were reported by Seleiman et al. (2022) and Bhuvaneshwari and Singh (2015), who found that combinations of Azolla with inorganic fertilizers can not only supply plants with adequate amounts of nitrogen, but also facilitate better use of these nutrients through the mineralization process, resulting in increased productivity. According to this finding, increased nutrient availability positively affects the overall amount of N absorbed by plants. The availability of N in the soil determines the amount of nitrogen that can be absorbed by plants. Additionally, Das et al. (2010) stated that applying a half inorganic fertilizer and half Azolla (5 t ha<sup>-1</sup>) increased soil fertility and significantly increased NPK uptake values in corn plants when compared to using recommended inorganic fertilizer doses. However, when compared to the application of urea alone, the addition of Azolla reduced N loss in rice plants and enhanced both N uptake and N-use efficiency (Seleiman et al., 2022). Banerjee et al. (2015) found a similar total N uptake (shoot plus fruit) increase of more than two to threefold depending on the level of N, consistent with the present results. Across both locations, the higher application of Azolla with inorganic N rates (Azolla rate of 15 t ha<sup>-1</sup>+138 kg N ha<sup>-1</sup>) resulted in higher nitrogen uptakes of tomato plants. These findings are consistent with (Setyono and Akbae, 2023), who discovered

that the application of Azolla treatment was 6,800 kg ha<sup>-1</sup>, which was higher at all levels of nitrogen use.

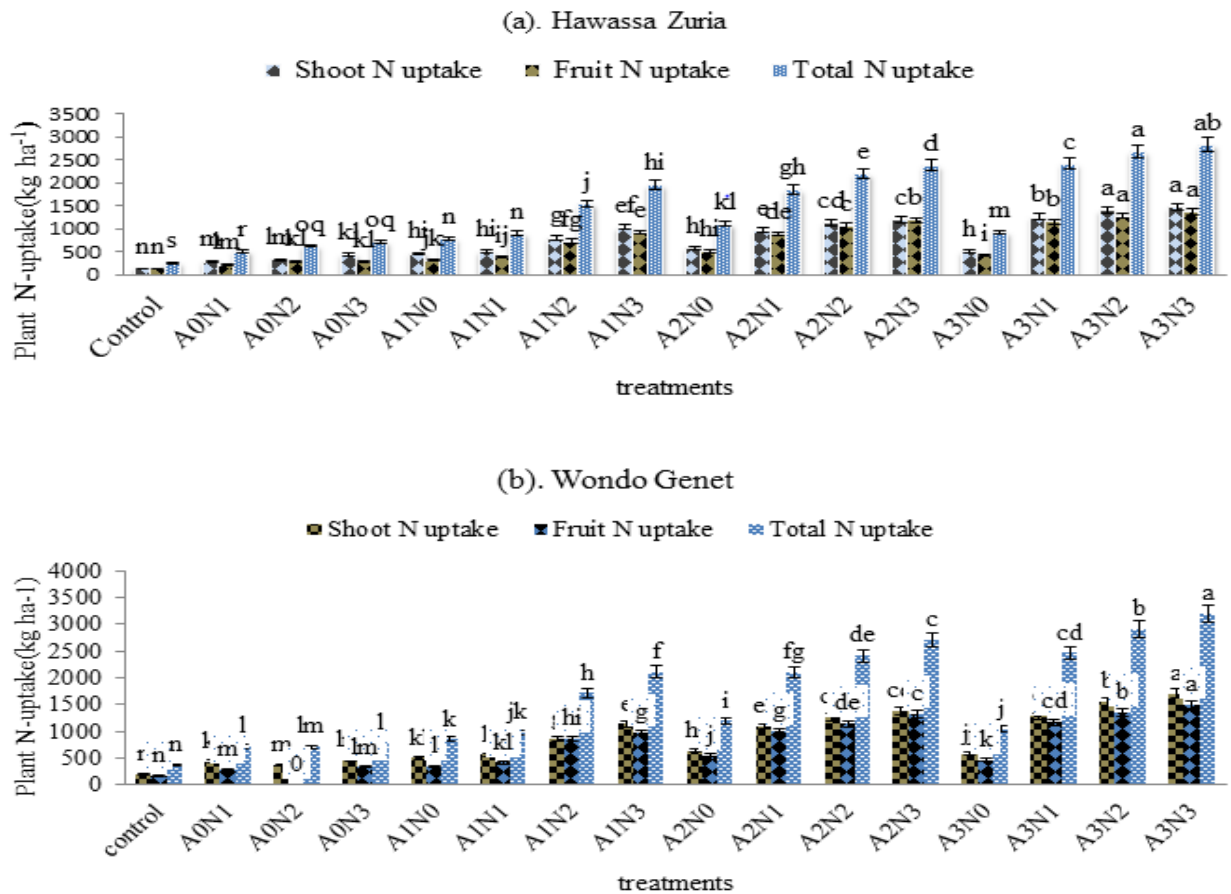


Figure 4.3. Shows the combined effects of Azolla dry biomass and inorganic N fertilizers on tomato shoot, fruit and total plant N-uptake at (a). Hawassa Zuria and (b). Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD). A0= 0; A1= 5; A2= 10; A3= 15 t Azolla ha<sup>-1</sup> and N0= 0; N1=46; N2= 92; N3= 138 kg N ha<sup>-1</sup>.

### **4.3.7. Nitrogen use efficiency parameters as influenced by Azolla dry biomass and N fertilizers**

#### **4.3.7.1. Agronomic use efficiency of nitrogen**

The combined two years of data (2022/23 and 2023/24) in both locations, the applications of Azolla dry biomass combined with inorganic N fertilizers resulted in significantly ( $P \leq 0.05$ ) higher agronomic efficiency of N. The combination of A0+N1 (0 t ha<sup>-1</sup> of Azolla dry biomass rate with 46 kg N ha<sup>-1</sup>) showed the highest efficiency (Figures 4.4a and b). There was variation in the agronomic efficiency of nitrogen when using Azolla dry biomass alone or inorganic nitrogen alone. However, the combined application of Azolla at 5 t ha<sup>-1</sup> + 92 kg N ha<sup>-1</sup> improved nitrogen use efficiency by more than 69% compared to the combination of Azolla dry biomass at 10 t ha<sup>-1</sup> + 92 kg N ha<sup>-1</sup>. Higher nitrogen rates were found to significantly reduce nitrogen uptake and use efficiency. The results showed that the rate of N uptake did not increase linearly with the amount of nitrogen applied.

In the Hawassa Zuria district, it showed that the agronomic use efficiency of different forms of nitrogen ranged from 4.91 to 142.39 kg kg<sup>-1</sup> (Figures 4.4a and b). The highest AE (142.39 kg kg<sup>-1</sup>) in Hawassa Zuria was achieved with the Azolla 0+46 kg ha<sup>-1</sup> of N-treated plot (Figure 4.4a). These results align with Winnie et al. (2018), who found that lower levels of N result in higher agronomic use efficiency of nitrogen. Among the two sources, the highest agronomic efficiency (AE) was observed in the Azolla 0+46 kg ha<sup>-1</sup> of N treated plot, followed by the Azolla 0+92 kg ha<sup>-1</sup> of N treated plot. In the Hawassa Zuria location, the lowest agronomic use efficiency of nitrogen (AE) was obtained from an Azolla dry biomass treatment of 15 t ha<sup>-1</sup> + 0 kg ha<sup>-1</sup> of N (4.91 kg kg<sup>-1</sup>) (Figure 4.4a). In the Wondo Genet location, the agronomic use efficiency ranged from 3.48 to 139.35 kg kg<sup>-1</sup> (Figure 4.4 b). The highest agronomic efficiency of 139.35 kg kg<sup>-1</sup> was obtained from the 46 kg ha<sup>-1</sup> treatment (Figure 4.4 b) at the Wondo Genet location, while the lowest AE of nitrogen was obtained from the Azolla dry biomass 15 t ha<sup>-1</sup> treatment (3.48 kg ha<sup>-1</sup>) (Figure 4.4 b). The present study results show that at minimum, agronomic efficiency tends to be at high nitrogen rates.

Across locations, the agronomic efficiency data revealed that completely inorganic nitrogen performed better than Azolla or any other mix of Azolla and inorganic nitrogen. Nitrogen use efficiency (NUE) decreases as nitrogen levels increase for potato crops (Banerjee et al. (2015).

These findings are consistent with Pilli et al. (2019), who found that the agronomic effectiveness of nitrogen in tomato crops showed that completely inorganic N performed better than completely organic fertilizers or other organic-inorganic nitrogen fertilizer combinations. Furthermore, this supports the findings of Abdelkader et al. (2023), who discovered that wheat harvests with high nitrogen levels often had low agronomic efficacy. Nitrogen utilization efficiency declined significantly as N fertilizer rates increased in both sites; these findings are consistent with many other N fertilizer rate studies (Hochmuth and Hanlon, 2014; Zotarelli et al., 2009). This decrease could be related to the increased availability of nitrogen. When plants acquire more nitrogen than they require, they are able to absorb and utilize the surplus nitrogen more efficiently, resulting in increased nitrogen utilization efficiency. The agronomic usage efficiency of both cropping seasons across locations demonstrated that inorganic nitrogen outperformed Azolla or any combination of Azolla and inorganic nitrogen. Similar findings were reported by Pilli et al. (2019), who found that the relative agronomic use efficiency of inorganic nitrogen was better than organic nitrogen or a combination of inorganic and organic fertilizers in the tomato crop.

In Hawassa Zuria, the agronomic efficiency of applied nitrogen declined as N levels rose for all cultivars with values of 39.26 kg kg<sup>-1</sup>. Higher agronomic effectiveness of applied nitrogen was noted in the co-application of Azolla with 50% lowering of N + 30 kg P ha<sup>-1</sup>, 30 kg K ha<sup>-1</sup>, and 20 kg S ha<sup>-1</sup>. A decreasing trend in nitrogen agronomic efficiency was reported with increasing N levels from 30 to 120 kg ha<sup>-1</sup> (Arduini et al., 2006). In the Hawassa Zuria location, the control had the lowest agronomic efficiency of 4.68 kg kg<sup>-1</sup> when given 15 t ha<sup>-1</sup> of Azolla (Figure 4.4a). This result is most likely due to the accumulation of soil nutrient reserves and the quick breakdown of Azolla, which is impacted by reduced C/N ratios, which provide abundant nutrients, notably N (Li et al., 2019; Zhou et al., 2021; Marzouk et al., 2024). Nitrogen agronomic efficiency decreased as N levels grew from 30 to 120 kg ha<sup>-1</sup> (Belete et al., 2018). According to Ghadirnezhad Shide et al. (2024), attempts to enhance nitrogen use efficiency will be critical to decreasing these negative impacts through improved agronomic techniques.

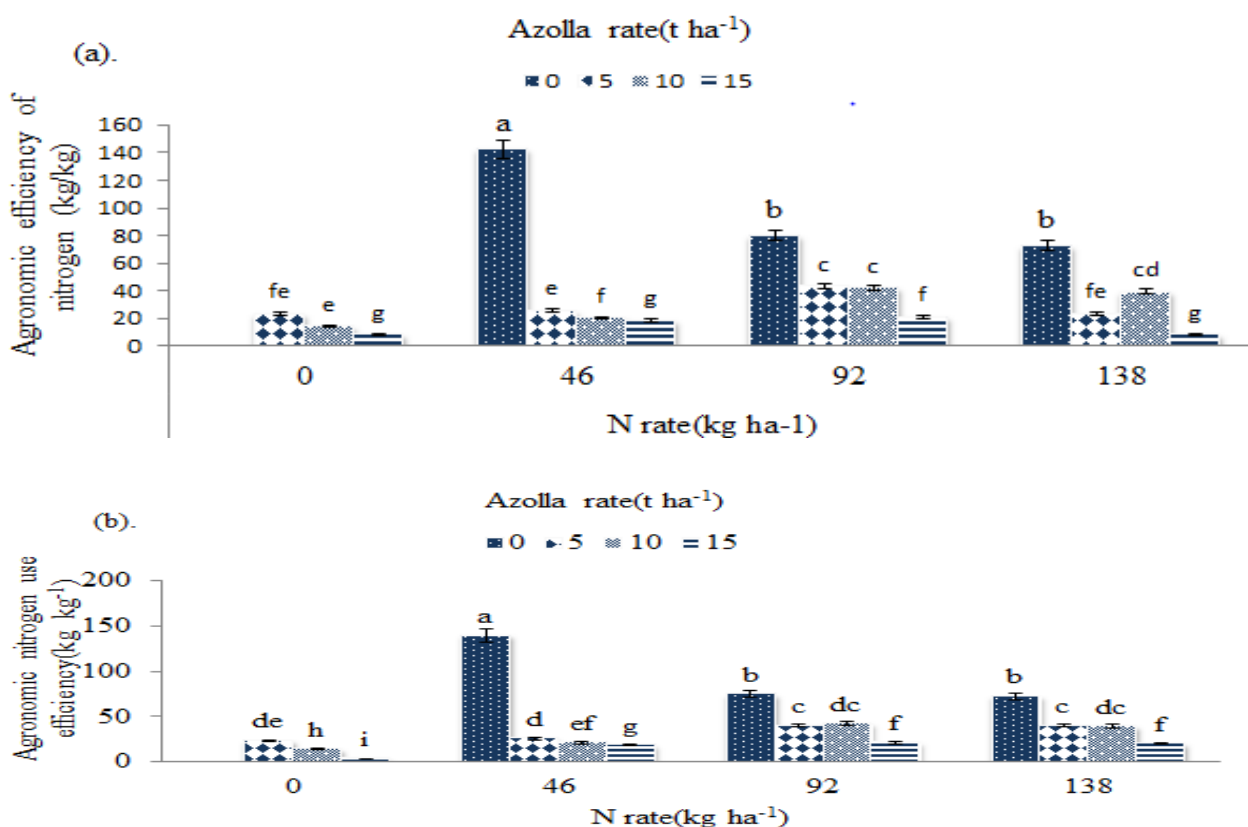


Figure 4.4. Interaction effect between Azolla dry biomass and inorganic N fertilizers on agronomic nitrogen use efficiency (AE) of (a) Hawassa Zuria (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

#### 4.3.7.2. Agro- Physiological efficiency of nitrogen

In the Hawassa Zuria and Wondo Genet districts, agro-physiological efficiency (APE) varied significantly with the combination of Azolla and inorganic N fertilizer rates, with significant two-way interactions (Figures 4.5a and b). A combined two-year analysis of data revealed that the Hawassa Zuria district had the highest agro-physiological efficiency ( $22.67 \text{ kg kg}^{-1}$ ) with an Azolla dry biomass rate of  $0 \text{ t ha}^{-1}$  and  $46 \text{ kg ha}^{-1}$  of Azolla (Figure 4.5a). The maximum APE ( $23.80 \text{ kg kg}^{-1}$ ) was reported in the Wondo Genet district when  $0 \text{ t ha}^{-1}$  of Azolla dry biomass and  $138 \text{ kg N ha}^{-1}$  were applied together (Figures 4.5b). This trend was similar to agronomic use efficiency, indicating low APE at high N levels in both the Hawassa Zuria and Wondo Genet locations, where APE reached  $4.67 \text{ kg kg}^{-1}$  and  $3.8367 \text{ kg kg}^{-1}$ , respectively, with the addition of  $15 \text{ t ha}^{-1}$  of Azolla (Figures 4.5a and b).

In the Hawassa Zuria location, the application of inorganic N ( $46 \text{ kg N ha}^{-1}$ ) raised N agro-physiological use efficiency (APE) by 5% to 79% when compared to the control group. In

Wondo Genet, the application of 138 kg N ha<sup>-1</sup> enhanced the PE of N by 17% to 84% when compared to other adjustments. The application of 46 kg N ha<sup>-1</sup> resulted in increased PE, possibly due to reduced N absorption compared to other amendments. Increased N levels decreased NUE in tomatoes. Banerjee et al. (2015) revealed similar findings on N physiological use efficiency (APE); however, the present results were lower than those of Grzebisz et al. (2015) and Trehan (2009). The authors suggested that at high N uptake, the agronomic efficiency (AE) decreases, possibly due to luxury consumption of N that does not contribute to physiological processes in the potato crop (Tsegaye, 2017). According to Safriyani et al. (2020), increased nitrogen application typically results in decreased agro-physiological efficiency, utilization efficiency, and apparent recovery efficiency. N physiological efficiency declined with increasing N fertilizer use, according to Eagle et al. (2001). The stage of plant growth determines the amount of nitrogen required and absorbed; an excess of nitrogen would disrupt rice growth and ecosystem stability, raising production costs (Zheng et al. 2007). The combined application of 86 kg N ha<sup>-1</sup> and 1000 kg Azolla ha<sup>-1</sup> also improved nitrogen fertilizer agro-physiological efficiency (APE), agronomic efficiency (AE), utilization efficiency (UE), and nitrogen efficiency ratio (NER), according to Safriyani et al. (2020), who also reported similar results.

The difference among the changes in APE obtained in this study was in agreement with the study by Belete et al. (2018), who reported that physiological use efficiency depends on genotypes. The maximum and minimum values of this parameter were recorded at the lowest and highest N rates, respectively, for both locations. In general, the agro-physiological efficiency of the treatments showed a value less than 60 kg kg<sup>-1</sup>, where this value is described as low, indicating a less managed system (Gauer et al., 1992).

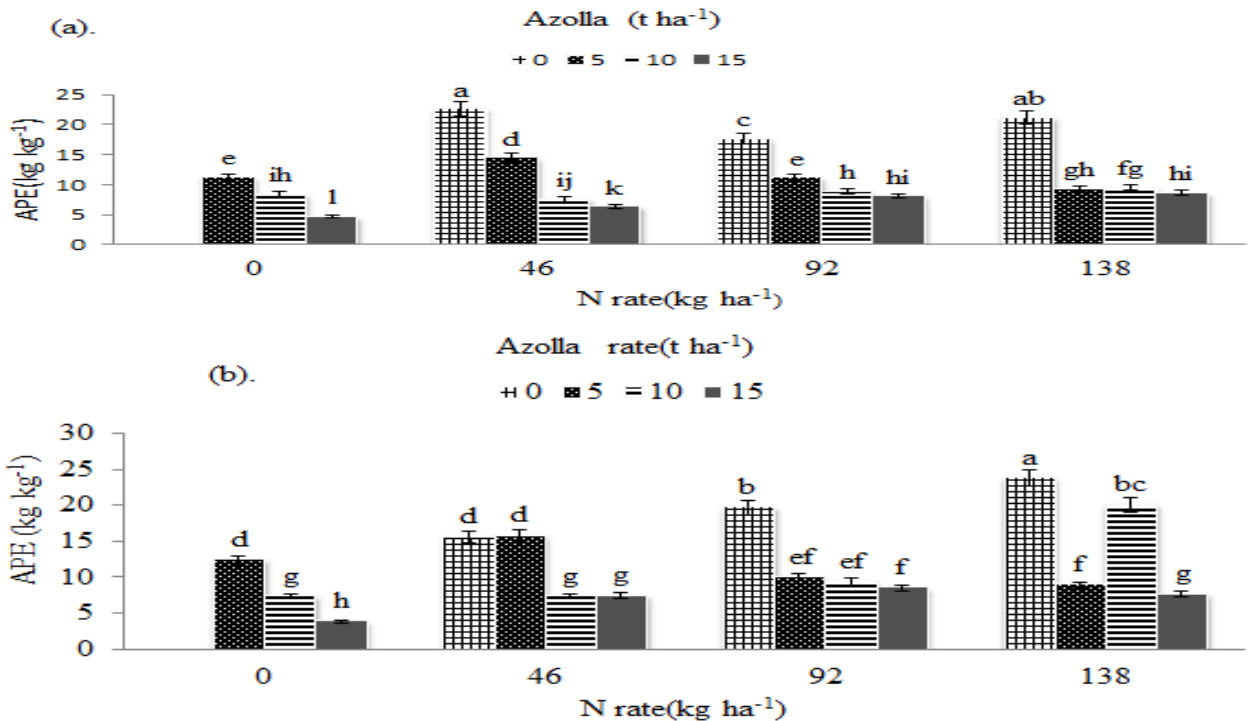


Figure 4.5. Interaction effect between Azolla dry biomass and inorganic N fertilizers on Agro-physiological nitrogen use efficiency (APE) of (a) Hawassa Zuria (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

#### 4.3.7.3. Apparent nitrogen recovery efficiency

Combined two years data (2022/23 and 2023/24) in both the Hawassa Zuria and Wondo Genet locations, the analysis of variance indicated that the apparent recovery efficiency (ARE) was significantly ( $P \leq 0.05$ ) influenced by the interaction of Azolla dry biomass rate and inorganic N rate (Figure 4.6a and b). Combined two years of data at the Hawassa Zuria and Wondo Genet locations, and the highest apparent recovery efficiency (ARE) of nitrogen was achieved in plots treated with inorganic nitrogen alone (46 kg N ha<sup>-1</sup>) (Figure 4.6a and b). Additionally, the plots that only used Azolla had a much lower nitrogen recovery efficiency compared to those that used both Azolla and inorganic nitrogen. In the Hawassa Zuria and Wondo Genet locations, the highest nitrogen recovery efficiency was 627.91% and 895.80%, respectively (Figure 4.6a and b). In both locations, the lowest apparent recovery efficiency was recorded with the sole application of Azolla dry biomass at a rate of 15 t ha<sup>-1</sup>. The trend showed that in the sole application of inorganic nitrogen, apparent recovery efficiency decreased with an increased N supply rate. However, in the combined application of Azolla with inorganic N fertilizers, the apparent recovery efficiency of nitrogen irregular system with an increased supply rate in both cropping locations (Figure 4.6a and b). The N recovery trend is similar to that of Tsegaye (2017) and

Banerjee et al. (2015), who discovered N recovery efficiency greater than 100%, ranging from 112 to 272% in potato crops. Jalpa et al. (2020) state that a tomato crop with a high apparent recovery has efficiently absorbed and used a sizable amount of the supplied nutrient, such as nitrogen (N), leading to higher yields, better nutrient uptake, and less environmental nutrient waste. Additionally, similar finding was reported by Scholberg et al. (2000), the decrease in apparent recovery efficiency with an increase in N fertilizers in tomato crop. An apparent recovery efficiency value of zero indicates that nitrogen accumulation in the unfertilized plant did not differ from that in the fertilized plant (Mengel et al., 2006).

The apparent nitrogen recovery efficiency is obtained by aligning plant nitrogen demand with the amount of nitrogen supplied from applied sources (Olsen, 1954). Similarly, Haile et al. (2012) discovered a decline in nitrogen utilization efficiency as nitrogen rates increased in the wheat crop under study. The study also found that as nitrogen content grew from 120 to 360 kg N ha<sup>-1</sup> in both growth years, all bread wheat types apparent nitrogen recovery efficiency (ARE) decreased. Kidanu et al. (2000) also discovered that wheat enhanced its ARE by 110 kg N ha<sup>-1</sup>, compared to 60 and 85 kg N ha<sup>-1</sup>. By comparison, bread wheat exhibited the highest N usage efficiency (39.27%) at the lowest nitrogen rate of 30 kg N ha<sup>-1</sup> and the lowest (27.10%) at a nitrogen rate of 120 kg N ha<sup>-1</sup>, according to Haile et al. (2012). The highest apparent nitrogen recovery efficiency was 39.27% (Haile et al., 2012), 65.8% (Kidanu et al., 2000), and 46.8% (Lemaire et al., 2004) according to research done in Ethiopia. Nonetheless, it is common to see apparent recovery N efficiency values between 50% and 80%, which indicate a well-managed system (Gauer et al., 1992).

Additionally, similar findings were reported by Rodrigues et al. (2021) on another crop (oats). They stated that in the non-amended (mineral) plots, apparent nitrogen recovery tended to decrease from the low to the higher nitrogen rates. In the final balance, after the second growing season of oats, the average values were 62.3%, 43.5%, and 46.6% in the N50, N100, and N200 treatments, respectively. The apparent recovery efficiency of N is a critical statistic that assesses how well plants recover and use nitrogen from applied fertilizers (Congreves et al., 2021). It provides insight into crop N uptake efficiency, which is critical for optimizing agricultural operations and achieving long-term nutrient management objectives. In general, higher AREN levels indicate more efficient nitrogen utilization by the crop. According to Marzouk (et al, 2024), the co-application of Azolla, rice straw, full and 50% reduced N, 30 kg P ha<sup>-1</sup>, and 20 kg S

ha<sup>-1</sup> led in a better percentage of N recovery efficiency. According to You et al. (2023), higher nitrogen recovery efficiency values were observed after the application of enhanced efficiency fertilizers.

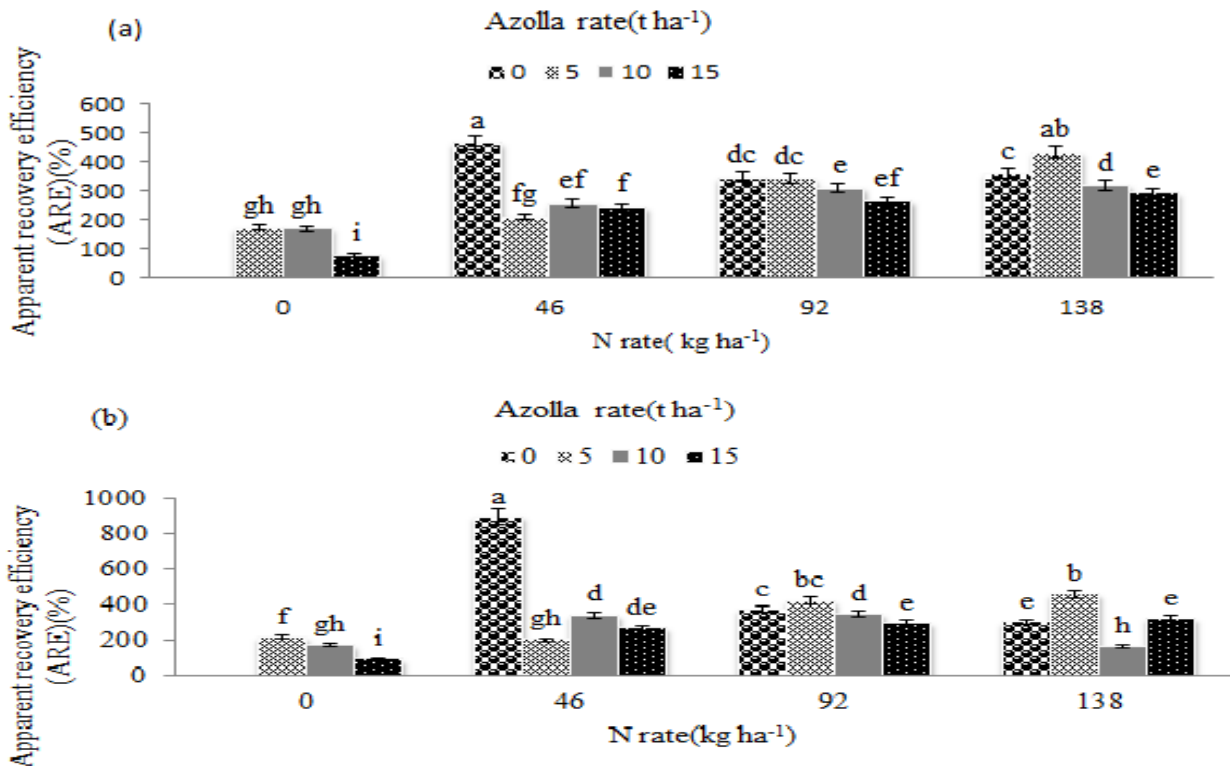


Figure 4.6. Interaction effect between Azolla dry biomass and inorganic N fertilizers on apparent nitrogen recovery efficiency (ARE) of (a) Hawassa Zuria (b) Wondo Genet districts. Unlike letter(s), imply significance difference between treatments at  $P < 0.05$  (LSD).

#### 4.4. Conclusions

This two-year field study conducted in the Hawassa Zuria and Wondo Genet districts of Sidama region demonstrated that integrating Azolla dry biomass with inorganic N fertilizer significantly improves soil fertility, tomato growth, and yield of tomatoes under irrigated conditions. Azolla, serving as an organic nitrogen source, enhanced soil nutrient status and, when combined with inorganic nitrogen, produced synergistic effects that boosted key yield components such as the number of clusters per plant, and fruits per cluster, and overall fruit yield. The highest marketable tomato yields were recorded with the combined application of 15 t ha<sup>-1</sup> Azolla dry biomass and 138 kg N ha<sup>-1</sup> inorganic nitrogen, highlighting the value of this integrated nutrient management approach. Control plots without any nutrient inputs consistently showed the lowest yields, emphasizing the importance of proper fertilization for sustainable tomato production in the

region. The combined application of Azolla dry biomass  $15 \text{ t ha}^{-1}$  and  $138 \text{ kg N ha}^{-1}$  led to the highest fruit N uptake ( $1093.48 \text{ kg ha}^{-1}$  in Hawassa Zuria and  $1486.94 \text{ kg ha}^{-1}$  in Wondo Genet), while the control treatment had the lowest. Based on these findings, farmers in the study areas are recommended to adopt the combined use of  $15 \text{ t ha}^{-1}$  Azolla dry biomass and  $138 \text{ kg ha}^{-1}$  inorganic nitrogen fertilizer to sustainably enhance tomato productivity under field conditions. Agricultural extension services should actively promote Azolla cultivation and its integration with inorganic fertilizers as a cost-effective and environmentally friendly strategy to improve soil fertility and crop yield. Further research is essential to evaluate the long-term effects of this integrated nutrient management on soil microbial communities, nutrient cycling, and environmental sustainability. Additionally, expanding similar investigations across diverse crops and agroecological zones will help confirm the scalability and broader applicability of integrating Azolla with inorganic nitrogen fertilizers as a sustainable agricultural practice.

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## CHAPTER FIVE

### 5. EFFECTS OF AZOLLA DRY BIOMASS ON SOIL NITROGEN MINERALIZATION IN SOILS COLLECTED FROM HAWASSA ZURIA AND WONDO GENET DISTRICTS INCUBATED UNDER LABORATORY CONDITIONS

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#### ABSTRACT

*Understanding the process of soil nitrogen (N) mineralization in response to different amendments is essential for improving nitrogen use efficiency and crop productivity. The present study aims to determine the effects of Azolla dry biomass and incubation periods on soil nitrogen mineralization under laboratory settings. The experiment was conducted in the soil laboratory at College of Agriculture, Hawassa University. Soil samples were collected from Hawassa Zuria and Wondo Genet districts and incubated for 56 days at dark 25°C with in plastic jar. Four levels of Azolla (0, 15, 30, and 45 g kg<sup>-1</sup> of soil) and eight incubation durations (0, 7, 14, 21, 28, 42, 49, and 56 days) were arranged in a factorial order and laid out in a completely randomized design (CRD) with three replications. Total available NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations were measured every seventh day using standard laboratory procedures. This study found significant interactions (P<0.001) between the effects of Azolla levels and incubation period under laboratory conditions on total mineralized N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N. The highest nitrogen mineralization occurred after 42 days of incubation with 45 g kg<sup>-1</sup> of Azolla in soils from both locations (Hawassa Zuria and Wondo Genet). The highest total mineralized N was found after 42 days of incubation (39.88 and 48.57 mg kg<sup>-1</sup> soil, respectively). In contrast, in both districts the control treatment had the lowest total mineral N level (1.33 and 1.94 mg kg<sup>-1</sup>, respectively). Nitrogen mineralization differed significantly among treatments (P<0.001). The use of Azolla greatly increased the rate of nitrogen mineralization in the soil, potentially reducing the demand for inorganic nitrogen fertilizer. This indicates that Azolla can improve crop nutrient availability by enhancing soil nitrogen content. However, further field trials are necessary to confirm these laboratory results and provide practical recommendations for field applications, especially regarding optimal timing for N mineralization in crop production systems.*

**Keywords:** Ammonium; Crop productivity; Incubation; Mineralization Potential; Nitrogen use efficiency; Organic fertilizer

## 5.1. Introduction

Nitrogen (N) is one of the most challenging nutrients to manage in organic crop production because its uptake relies on the availability of N in the soil. Organic matter must first decompose into nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ) ions in order for plants to absorb them. According to Sukor et al. (2013), nutrient management in organic systems must take into consideration how plants, soil organic matter (SOM), and soil organisms affect the availability of nitrogen. As a result of several nitrogen loss mechanisms, such as oxidized and reduced layers, floodwater, runoff, leaching, and biological changes, managing nitrogen in soil is complicated. For crops to be available without causing major environmental harm, careful management is required (Anggria et al., 2012).

Soil nitrogen mineralization plays a critical role in nitrogen availability and balance in soils (Wang et al., 2019). It is a key step in the transformation of organic nitrogen into inorganic forms like ammonium ( $\text{NH}_4^+\text{-N}$ ) and nitrate ( $\text{NO}_3^-\text{-N}$ ), or transitional forms like soluble organic nitrogen (SON), which are later mineralized. This biological process depends heavily on organic matter composition and soil microbial activity (Zhang et al., 2017). High nitrogen content and low carbon-to-nitrogen ratios favor plant growth by enhancing nitrogen availability (Manojlovic et al., 2010). The application of Azolla provides natural source nutrients and has great potential to improve soil health and increase yield sustainability. The use of Azolla as an organic fertilizer is emerging as a sustainable alternative to inorganic fertilizers in agriculture (Santhiya and Jeeva, 2022; Zhao et al., 2024). Research indicates that integrating organic fertilizers with inorganic fertilizers can increase crop yields by 10%–40% and reduce the use of inorganic fertilizers by 25%–30% (Pal et al., 2015). Azolla improves nitrogen use efficiency (NUE) in paddy rice systems by enhancing biological nitrogen fixation (Santhiya and Jeeva, 2022). It also supports nutrient uptake and promotes soil fertility while minimizing environmental impact (Kumar et al., 2020).

Azolla's decomposition rate in soil depends on C/N ratio, temperature, and properties. It improves aggregation, structure, permeability, and water retention; reduces evapotranspiration; increases soil nitrogen and phosphorus; and lowers pH (Abd-el Rasoul et al., 2004). The significant increase in total nitrogen (TN) levels in Azolla-fertilized soils is primarily due to Azolla's ability to fix atmospheric nitrogen. This process occurs through a symbiotic relationship with nitrogen-

fixing cyanobacteria. As a result, Azolla mineralization enhances nitrogen availability in the soil, making it an effective solution for improving soil fertility and supporting crop growth (Marzouk et al., 2024).

Azolla typically has a low carbon-to-nitrogen (C/N) ratio, which enables it to mineralize faster than many other organic fertilizers. In waterlogged soils, about 60-80% of Azolla's nitrogen can be mineralized within two weeks (Jama et al., 2023). However, in tropical climates, this process may take 30-60 days, while in temperate climates it can extend beyond 60 days (Bhuvaneshwari and Kumar, 2013; Jama et al., 2023). In the past, soil incubation experiments have been done to estimate the potentially mineralizable N in soils (Stanford and Smith, 1972). In various studies, the course of mineralization for several organic fertilizers has been studied under controlled laboratory conditions (Griffin and Honeycutt, 2000). Many studies have been conducted on soil nitrogen mineralization of organic fertilizers, but there is little research specifically on the effects of Azolla dry biomass on soil N mineralization at different incubation periods. There is not enough literature that specifically addresses how Azolla, as a source of organic nitrogen, could affect soil nitrogen mineralization rates over different incubation periods. Despite studies on nitrogen mineralization for various organic fertilizers, research specifically on the mineralization of nitrogen in Azolla dry biomass remains limited. Therefore, the goal of this study was to determine the effects of Azolla dry biomass rates and incubation periods on nitrogen mineralization under controlled settings. The findings will provide insights into the potential of Azolla dry biomass for improving soil fertility and nitrogen availability in sustainable agricultural practices.

## **5.2. Materials and Methods**

### **5.2.1 Description of the study areas**

The experiment was carried out at Hawassa University Soil Science Laboratory, where the temperature and relative humidity were maintained at 25°C and 70.25%, respectively. The soils for the experiment were collected from Hawassa Zuria and Wondo Genet districts. The soil samples were collected from Hawassa Zuria is located at 7°0'13.7" to 7°48'11.5" N and 38°21'21.1" to 38°63'30.5" E, with an average elevation of 1712 meters above sea level. It has an average annual rainfall of 952 mm and mean minimum and maximum temperatures of 12.1°C and 26.7°C, respectively (Hawassa Meteorological Center, 2018). The soil samples collected from Wondo Genet district is located from 6°16' to 7°45' N and 38°31' to 38°63' E. The district

altitude ranges from 1001 to 1780 meters above sea level, with an annual rainfall of 801 to 1221 mm and temperatures between 17.6°C and 22.5°C. Before the incubation experiment, the soil analysis results for the Hawassa Zuria district showed that the soil properties included a sandy loam texture, a soil pH of 8.62, organic carbon content of 2.16%, and total nitrogen content of 0.16%. In comparison, the soil characteristics of the Wondo Genet location included a sandy clay loam texture, a soil pH of 7.21, organic carbon content of 4.28%, and total nitrogen content of 0.3%. Before conducting the experiment the analysis of Azolla resulted in a pH of 7.02, total N of 5.08% and organic carbon of 48%.

### **5.2.2. Soil Sample collection and preparation**

Soil samples were collected from the 0–20 cm surface layer from the Wondo Genet (Aruma) and Hawassa Zuria (Jara Gelalicha) kebeles. At each location, several subsamples or individual auger samples were randomly taken from different positions in an agricultural field in a zigzag pattern, and then mixed to form a composite sample, ensuring a representative and accurate soil of each area. The composite samples were sieved to 2.0 mm for uniform particle size before incubation. The soil was collected from the cultivated fields at a depth of 0 to 20 cm from the selected locations and incubated for 56 consecutive days after the application of Azolla. After incubation, the amount of nitrogen mineralized from the soil (ammonium and nitrate) was measured after 7 days. All the samples were subsequently transported to the Hawassa University Soil Science Laboratory, School of Plant and Horticultural Sciences, where sieving was carried out using a 2-mm sieve for all the nutrients except for organic carbon and total nitrogen, which were sieved through a 0.5 mm mesh size.

### **5.2.3. Experimental design, treatments, and procedures**

The nitrogen mineralization experiment consisted of 32 treatments, four dry Azolla biomass levels (0, 15, 30, and 45 g kg<sup>-1</sup> of soil) with eight incubation periods (0, 7, 14, 21, 28, 42, 49, and 56 days), and were laid out in completely randomized design (CRD) with three replications. A total of 96 jars for each location of soil were used to carry out the experiment. The volume of each plastic jar was 1500 ml, and each jar contained 1000 g of finely ground, air-dried soil (sieved through a 2-mm mesh). The soil was weighed and transferred into the plastic jars for all treatments. Azolla dry biomass was weighed and added to all treatments except for the control group. After the treatments were added, all the jars were weighed, and their weights were recorded. The soil-filled plastic jars were then covered with aluminum foil, with three small holes

on the top to allow for oxygen exchange and the jars were incubated under controlled conditions in the dark at 25°C. Soil moisture was adjusted at 60% field capacity for 56 incubation days. The study aimed to evaluate nitrogen mineralization over time, with samples analyzed at each interval. Ammonium ( $\text{NH}_4^+$ ) extraction was performed on 20 g of soil using 50 mL of 2 M KCl. The mixture was shaken for one hour on a reciprocal shaker and then filtered. The resulting solution was analyzed for  $\text{NH}_4^+$  using a visible spectrophotometer at 636 nm. For nitrate ions ( $\text{NO}_3^-$ ), extraction was done using 0.01 M  $\text{CaCl}_2$ , and measurements were taken using an ultraviolet spectrophotometer at 210 and 275 nm. Measurements of mineral nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) were performed at regular intervals (0, 7, 14, 21, 28, 42, 49, and 56 days) to assess nitrogen mineralization and release from Azolla. This process allowed for the evaluation of nitrogen availability throughout the incubation period and the effectiveness of different Azolla concentrations on enhancing nitrogen mineralization (Anggria et al., 2012).

#### **5.2.4. Soil analysis from the pre-incubation experiment**

The bulk density of the soil was determined using the cylindrical core method (Arshad et al., 1996). Soil texture was analyzed by the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was measured with a pH meter in water at a 1:2.5 soil-to-water solution ratio (Van Reeuwijk, 1992). The organic carbon (OC) content was measured using the Walkley-Black wet digestion method, and total nitrogen was determined via the Kjeldahl method (Jackson, 1958).

#### **5.2.5. Soil incubation and sampling procedure**

The incubation methods used in this study were performed according to (Kaleem et al., 2015). The samples were incubated in the laboratory, after which the samples were analyzed in accordance with a completely randomized design (CRD) with three replicates. The moisture content of the mixture was maintained at 60% water holding capacity and incubated at 25°C in dark for 56 days. Soil samples were taken at seven-day intervals from the incubated soils for the subsequent eight weeks (56 days) after the Azolla were incorporated into the soil. Soil samples were collected at soil depths of 0–20 cm using an auger from different randomly two selected districts. The 0-20 cm depth represents the topsoil surface in most agricultural soils, making it the ideal range for collecting soil samples for N mineralization. The time intervals involved 8 incubation periods, that is, 0, 7, 14, 21, 28, 42, 49, and 56 days (after the application of the amendments). Soil sampling was carried out after 0, 7, 14, 21, 28, 42, 49, and 56 days of incubation (for each sampling time, replicate treatments were taken for analysis). Sampling

intervals assuming that every 7 days, Azolla releases a certain amount of inorganic nitrogen ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) into the soil system.

#### **5.2.6. Azolla source, preparation and application**

Azolla biomass was received from Hawassa University's College of Agriculture, School of Animal and Range Sciences. A sufficient amount of Azolla biomass was obtained for the experiment, which was gently washed with tap water and dried with tissue paper. The dried Azolla was carefully weighed using a sensitive balance, the amount varying according to the treatment rate. It was then mixed with 1000 g of sieved soil for each treatment, except for the control group. The soil and dried Azolla mixture were placed in a plastic jars container with a volume of 1500 mL.

#### **5.2.7. Measurements of mineral nitrogen, and soil extraction and analysis**

The study aimed to evaluate nitrogen mineralization over time, with samples analyzed at each interval. Samples from all treatments were incubated at different intervals for analysis of ammonium-N ( $\text{NH}_4^+$ -N), nitrate-N ( $\text{NO}_3^-$ -N), and total mineral nitrogen ( $\text{NH}_4^+$ -N +  $\text{NO}_3^-$ -N). Subsequently, soil samples from each treatment were removed randomly from the incubator after different incubation periods. Ammonium ( $\text{NH}_4^+$ ) extraction was taken on 20 g of soil using 50 mL of 2 M KCl. The mixture was shaken for 1 hour on a reciprocal shaker and then filtered using Whatman No. 42 filter paper. The resulting solution was analyzed for  $\text{NH}_4^+$  using a visible spectrophotometer at 636 nm. For nitrate ions ( $\text{NO}_3^-$ ), extraction was done using 0.01 M  $\text{CaCl}_2$ , and measurements were taken using an ultraviolet spectrophotometer at 210 and 275 nm. Measurements of mineral nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) were performed at regular intervals (0, 7, 14, 21, 28, 42, 49, and 56 days) to assess nitrogen mineralization and release from Azolla. This process allowed for the evaluation of nitrogen availability throughout the incubation period and the effectiveness of different Azolla concentrations on enhancing nitrogen mineralization (Anggria et al., 2012). The C and N contents (total C and total N) of the soil were also monitored during incubation. For this purpose, a 10 g subsample of incubated soil was removed from each jar during each sampling period and placed in dictated Petri dishes to determine changes in total N and C with time. The soil organic carbon content was determined according to Nelson and Sommers (1982), while the total soil N content was determined using the Kjeldahl method (Bremner, 1982). The net cumulative mineralization of N expressed as the mineralization of N (or total mineral N) and the net cumulative N nitrified as  $\text{NO}_3^-$ -N accumulation at each sampling

time (t) were calculated as previously described (Griffin et al., 2005). This comprehensive approach provided insights into the dynamics of nitrogen availability in response to Azolla application during the incubation period.

The percentage of total N mineralized from each Azolla application at each sampling time was calculated as described by Azeez and Van Averbek (2010,) and Abbasi et al. (2007) as follows:

$$\% \text{ mineralization} = \frac{NM(\text{amended}) - NM(\text{control})}{N \text{ total (applied)}} \times 100$$

where NM is the total mineral N and N is the total N in the applied Azolla and N-fertilizer.

### 5.2.7. Nitrogen mineralization and N availability calculation

The net mineralization rates of N in the soil were determined by subtracting the final inorganic N (day 56) from the initial inorganic N (day 0) of the soil and dividing the resulting number by the number of days of incubation (Jama, 2018).

The nitrogen mineralization rate was calculated as follows:

$$\text{Net N mineralization} = (\text{NH}_4^+\text{-N treatment} + \text{NO}_3^-\text{-N treatment}) - (\text{NH}_4^+\text{-N control} + \text{NO}_3^-\text{-N control}) \dots\dots\dots (\text{Eqn. 1})$$

$$\text{Nitrogen availability \%} = (\text{net N mineralization/amount N applied as fertilizer}) \times 100 \dots\dots\dots (\text{Eqn. 2})$$

### 5.2.8. Data collection

The laboratory incubation data collected from all treatments consisted of several key measurements. Ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) concentrations were monitored throughout different incubation periods. Additionally, total nitrogen (TN) levels were measured to evaluate the overall nitrogen content in the soil. Soil organic carbon (SOC) content was also analyzed, as it plays a critical role in soil health and fertility. Together, these parameters provide a comprehensive understanding of how the treatments influence nitrogen mineralization and soil quality during the incubation process.

### **5.2.9. Data analysis**

The data obtained from the soil nitrogen mineralization analysis were subjected to analysis of variance (ANOVA) using the GLM (General Linear Model) of SAS version 9.4 statistical analysis software (SAS Institute, 2016). Data were separated using analysis of variance (ANOVA) and means were separated using the Least Significant Difference (LSD) test at a 5% level of probability was employed to separate treatment means where significant differences exist (Gomez and Gomez, 1984).

## **5.3. Results and Discussion**

### **5.3.1. Ammonium nitrogen ( $\text{NH}_4^+$ -N) release affected by Azolla during different incubation periods**

The interaction between Azolla application rates and incubation periods significantly influenced  $\text{NH}_4^+$ -N availability in soils at both study locations ( $p < 0.001$ ). Tables 5.1 and 5.2 present the mean  $\text{NH}_4^+$ -N concentrations at various incubation periods and Azolla application rates. On day 0,  $\text{NH}_4^+$ -N concentrations in soils treated with Azolla at rates of 0 (control), 15, 30, and 45  $\text{g kg}^{-1}$  were relatively low. However, over the incubation period,  $\text{NH}_4^+$ -N levels increased markedly, particularly with higher rates of Azolla. After 42 days of incubation, the highest  $\text{NH}_4^+$ -N concentrations were recorded in soils treated with 45  $\text{g kg}^{-1}$  Azolla, reaching peaks before declining in subsequent days. This pattern of increase from day 0 to day 42, followed by a decrease through day 56, was consistent across all treatments. Notably, the  $\text{NH}_4^+$ -N concentrations were lowest on day 0 and significantly higher on subsequent sampling days, highlighting the effect of Azolla application on nitrogen availability.

In both locations, the rate of Azolla significantly affected the availability of mineralized  $\text{NH}_4^+$ -N concentrations at different incubation periods and the concentration of ammonium was decreased trends after 42 day of incubation (Table 5.1 and 5.2). Similarly, (Jama et al., 2023) found that soil  $\text{NH}_4^+$ -N concentrations were considerably greater in the Azolla + Watanabe treatment than in the Azolla treatment on days 28, 56, 112, and 140. Azolla significantly affects the release of ammonium nitrogen ( $\text{NH}_4^+$ -N) during different incubation periods, with studies showing that as Azolla decomposes, it releases a substantial amount of readily available ammonium nitrogen, particularly in the early stages of incubation, due to the breakdown of its nitrogen-rich biomass, which can be readily utilized by plants like rice grown in flooded paddy fields (Xia et al., 2023). As the incubation period progresses, the rate of  $\text{NH}_4^+$ -N release from Azolla may slow down as

the readily available nitrogen is utilized by microbes or becomes incorporated into the soil organic matter (Ventura et al., 1992). Several authors have reported similar results on the impact of Azolla and inorganic N fertilizers on soil nitrogen mineralization for vegetable crops (Jama et al., 2023; Assuming-Brempong et al., 2008). Additionally, a similar result was reported by Roy et al. (2014), who found that soil type, incubation time, and the rate of organic material application influence nitrogen mineralization. According to our findings, the amount of nitrogen in Azolla-amended soils increased more with incubation time than in control soil.

Other researchers' findings were similar as well (Rahman et al., 2013; Dikinya et al., 2010; Vel Murugan et al., 2013). Azolla treatment at a dose of 10 t ha<sup>-1</sup> has a considerable effect on N-NH<sub>4</sub><sup>+</sup> dynamics in soil (Dewi et al., 2018). The composition of inorganic N is relatively simple, mainly consisting of NH<sub>4</sub><sup>+</sup> -N and NO<sub>3</sub><sup>-</sup> -N, which are the main available form of nitrogen in soil (Chang et al., 2023). Azolla significantly affects the release of ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) during different incubation periods. Studies show that as Azolla decomposes, it releases a substantial amount of readily available ammonium nitrogen, especially in the early stages of incubation. This is due to the breakdown of its nitrogen-rich biomass, which can be readily utilized by plants like rice grown in flooded paddy fields (Xia et al., 2023). As the incubation period progresses, the rate of NH<sub>4</sub><sup>+</sup> -N release from Azolla may slow down as the readily available nitrogen is utilized by microbes or becomes incorporated into the soil organic matter (Ventura et al., 1992). High ammonium(NH<sub>4</sub><sup>+</sup> -N) nitrogen mineralization rates were particularly evident between 42 - 49 days of incubation, especially in treatments with the highest Azolla application rates, consistent with findings that indicate optimal nitrogen mineralization occurs within 30-60 days of incubation (Assuming-Brempong et al., 2008). At both locations, the lowest ammonium concentration was zero for all Azolla treatments. In comparison, the NH<sub>4</sub><sup>+</sup> -N concentrations increased significantly with Azolla application, demonstrating the positive impact of organic fertilizers on the ammonification of organic nitrogen during the incubation periods. Organic nitrogen sources, such as plant and animal residues, can be transformed into inorganic forms through processes like ammonification and nitrification. This transformation follows the sequence: NH<sub>4</sub><sup>+</sup> → NH<sub>2</sub>OH → NOH → NO<sub>2</sub><sup>-</sup> → NO<sub>3</sub><sup>-</sup> (Anggria et al., 2012). Table 5.1 and 5.2 display the ammonium concentrations in soils from Hawassa Zuria and Wondo Genet, which initially showed low rates of mineralization. The highest mineralization rates were observed on day 42, with the following order of effectiveness: 45 g kg<sup>-1</sup> > 30 g kg<sup>-1</sup> > 15 g kg<sup>-1</sup> > control. Increased application rates of Azolla led to greater nitrogen mineralization. In contrast, Kalita et

al. (2023) reported that ammonium nitrogen levels peaked between days 10 and 40 during incubation, after which they gradually declined. After day 42,  $\text{NH}_4^+$ -N concentrations began to decline, consistent with other studies indicating that 60-80% of Azolla nitrogen mineralizes within two weeks in waterlogged soils (Ito et al., 1985). This decline may result from rapid immobilization by microorganisms, alongside nitrification and denitrification processes. In tropical climates, Azolla may fully mineralize in 30-60 days, while in temperate regions; this can extend beyond 60 days (Asuming-Brempong et al., 2008; Bhubaneswar & Kumar, 2013; Jama et al., 2023).

Table 5.1.  $\text{NH}_4^+$  -N contents ( $\text{mg kg}^{-1}$ ) of soils after different incubation periods in the laboratory from the Hawassa Zuria district

Dry Azolla rate( $\text{g kg}^{-1}$ )	Incubation periods							
	0	7	14	21	28	42	49	56
0	0.61 <sup>o</sup>	0.62 <sup>o</sup>	0.61 <sup>o</sup>	0.59 <sup>o</sup>	0.60 <sup>o</sup>	0.66 <sup>o</sup>	0.62 <sup>o</sup>	0.58 <sup>o</sup>
15	0.57 <sup>o</sup>	1.12 <sup>o</sup>	2.26 <sup>n</sup>	2.13 <sup>n</sup>	3.12 <sup>m</sup>	3.44 <sup>m</sup>	3.08 <sup>m</sup>	2.26 <sup>n</sup>
30	0.61 <sup>o</sup>	4.97 <sup>l</sup>	4.92 <sup>l</sup>	6.34 <sup>kj</sup>	6.71 <sup>ij</sup>	16.85 <sup>b</sup>	13.23 <sup>d</sup>	8.68 <sup>f</sup>
45	0.63 <sup>o</sup>	5.87 <sup>k</sup>	7.27 <sup>ih</sup>	7.76 <sup>gh</sup>	8.15 <sup>gf</sup>	20.27 <sup>a</sup>	14.41 <sup>c</sup>	11.44 <sup>e</sup>
<b>LSD(0.05)</b>	<b>0.77</b>							
<b>CV (%)</b>	<b>9.14</b>							

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

Table 5.2.  $\text{NH}_4^+$  -N contents ( $\text{mg kg}^{-1}$ ) of soils after different incubation periods in the laboratory the soil collected from Wondo Genet district

Dry Azolla rate( $\text{g kg}^{-1}$ )	Incubation periods							
	0	7	14	21	28	42	49	56
0	0.86 <sup>o</sup>	0.85 <sup>o</sup>	0.86 <sup>o</sup>	0.88 <sup>o</sup>	0.86 <sup>o</sup>	0.88 <sup>o</sup>	0.86 <sup>o</sup>	0.89 <sup>o</sup>
15	0.86 <sup>o</sup>	2.64 <sup>n</sup>	2.64 <sup>n</sup>	2.73 <sup>n</sup>	3.73 <sup>m</sup>	4.64 <sup>l</sup>	3.72 <sup>m</sup>	3.34 <sup>m</sup>
30	0.85 <sup>o</sup>	7.00 <sup>jk</sup>	7.34 <sup>ijk</sup>	8.15 <sup>hi</sup>	9.13 <sup>g</sup>	21.35 <sup>b</sup>	17.96 <sup>d</sup>	12.87 <sup>e</sup>
45	0.86 <sup>o</sup>	7.77 <sup>ij</sup>	8.85 <sup>hg</sup>	11.03 <sup>f</sup>	10.78 <sup>f</sup>	24.31 <sup>a</sup>	20.29 <sup>c</sup>	17.52 <sup>d</sup>

<b>LSD(0.05)</b>	<b>0.90</b>
<b>CV (%)</b>	<b>7.62</b>

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

### 5.3.2. Nitrate-N ( $\text{NO}_3^-$ N) release affected by Azolla at different incubation periods

In both locations, the concentrations of nitrate nitrogen were significantly affected ( $P < 0.001$ ) by the application of varying Azolla rates. The concentrations of  $\text{NO}_3^-$  N rose gradually between day 0 and day 42, but then began to fall. The concentrations of  $\text{NO}_3^-$ -N in the Hawassa Zuria and Wondo Genet locations decreased at the end of the incubation period (Table 5.3 and 5.4). Mineralization rates peaked on day 42, at which time the highest amount of  $\text{NO}_3^-$  -N was frequently recorded 42 days after the application of Azolla. After 42 days of incubation, the  $\text{NO}_3^-$  -N concentrations in the soil at both locations decreased in all the treatments and in the control (Table 5.3 and 5.4). The incubation periods studied can be arranged based on their impact on the  $\text{NO}_3^-$ -N content in the following order: zero < 7 < 14 < 21 < 28 < 42 > 49 > 56 days of incubation. The production of nitrate by nitrification is highly dependent on other N-transformation processes in the soil, especially the accumulation of nitrification substrates (i.e.,  $\text{NH}_4^+$ ). At both locations, the pattern of nitrate accumulation in response to the applied Azolla was similar to that of mineralization. In Hawassa Zuria, the accumulation of  $\text{NO}_3^-$ -N was 5.10, 17.89, and 19.61  $\text{mg kg}^{-1}$  soil relative to 0.69  $\text{mg NO}_3^-$  -N  $\text{kg}^{-1}$  in the control, showing that Azolla 15, 30, and 45  $\text{g kg}^{-1}$  accumulated  $\text{NO}_3^-$  -N almost seven times and more than seven times more than in the control soil without amendments, respectively (Table 5.3). At the Wondo Genet location, the accumulations of  $\text{NO}_3^-$  -N were 6.46, 20.89, and 24.26  $\text{mg kg}^{-1}$  soil relative to 1.05  $\text{mg kg}^{-1}$   $\text{NO}_3^-$  -N in the control, showing that the 15  $\text{g kg}^{-1}$  treatment resulted in a nearly six fold increase in  $\text{NO}_3^-$ -N and that the 30 and 45  $\text{g kg}^{-1}$  treatments resulted in more than six times more  $\text{NO}_3^-$ -N than did the control soil without amendments (Table 5.4). Similarly, the accumulation of  $\text{NO}_3^-$ -N in the Hawassa Zuria and Wondo Genet locations in soils treated with 45  $\text{g kg}^{-1}$  Azolla was 4 and 4.48 times greater than that in the control, respectively (Table 5.3 and 5.4). In the soil collected from the Hawassa Zuria and Wondo Genet sites, the maximum nitrate N content was recorded at the 45  $\text{g kg}^{-1}$  treatment (19.61 and 24.26  $\text{mg kg}^{-1}$ , respectively) at 42 days of incubation. At Hawassa Zuria, after 42 days of incubation, the proportions of total mineral N relative to  $\text{NO}_3^-$ -N were 52, 60, 51, and 49% of the total mineral N in the control group and 15,

30, and 45 g kg<sup>-1</sup>, respectively. At the Wondo Genet location, the proportions of total mineral N relative to NO<sub>3</sub><sup>-</sup>-N were 54, 58, 49, and 50%, respectively, of the total mineral N in the control group and 15, 30, and 45 g kg<sup>-1</sup>, respectively.

We found that the release of NO<sub>3</sub><sup>-</sup>-N mineralization was primarily influenced by the rate of Azolla and the number of days of incubation (Table 5.3 and 5.4). The concentration of NO<sub>3</sub><sup>-</sup>-N increased significantly with higher applications of Azolla during the 42-day incubation periods at both the Hawassa and Wondo Genet locations (Table 5.3 and 5.4). A similar finding was reported by Asuming-Brempong et al. (2008), who found that the release pattern of nitrate was low from day 0 to day 30 of incubation. From days 40 to 60, the release of nitrate N was high; resulting in high total N released and from day 10 to day 30, immobilization of nitrate N was observed for the dry Azolla. Glinski et al. (2007) reported that NO<sub>3</sub><sup>-</sup>-N concentrations increased near the end of the incubation period. The increase in nitrate-N concentration was increased with higher rates of Azolla application, as reported by other authors who studied the effects of Azolla on nitrate-N levels in vegetable crops (Jama et al., 2023). The most substantial difference in nitrate-N concentrations was observed at the 45 g kg<sup>-1</sup> Azolla treatment. This aligns with findings from Glinski et al. (2007), who also reported that NO<sub>3</sub><sup>-</sup>-N concentrations tend to rise towards the end of incubation. Moreover, the study found that nitrate concentrations increased with higher Azolla application rates and longer incubation periods. This aligns with the findings of Murugan and Swarnam (2013), who noted that nitrate levels decreased with a reduction in the application rates of manures and fertilizers. Additionally, the Azolla + Watanabe treatment showed significantly higher soil NO<sub>3</sub><sup>-</sup>-N concentrations than the Azolla treatment from day 7 through day 140 (Jama et al., 2023). Odhiambo (2010) also reported similar findings from his study on nitrogen release by green manure in different soil types.

Table 5.3. NO<sub>3</sub><sup>-</sup>-N contents (mg kg<sup>-1</sup>) of soils after different incubation periods in the laboratory from the Hawassa Zuria district

Dry Azolla rate(g kg <sup>-1</sup> )	Incubation periods							
	0	7	14	21	28	42	49	56
0	0.72 <sup>n</sup>	0.71 <sup>n</sup>	0.72 <sup>n</sup>	0.74 <sup>n</sup>	0.73 <sup>n</sup>	0.69 <sup>n</sup>	0.71 <sup>n</sup>	0.75 <sup>n</sup>
15	0.76 <sup>n</sup>	1.37 <sup>nm</sup>	2.65 <sup>ml</sup>	3.15 <sup>kl</sup>	4.36 <sup>jk</sup>	5.10 <sup>ji</sup>	3.85 <sup>jkl</sup>	3.14 <sup>kl</sup>

30	0.73 <sup>n</sup>	6.58 <sup>hi</sup>	7.27 <sup>hg</sup>	8.17 <sup>fg</sup>	8.96 <sup>fe</sup>	17.87 <sup>b</sup>	14.31 <sup>dc</sup>	9.77 <sup>e</sup>
45	0.70 <sup>n</sup>	7.41 <sup>hg</sup>	9.15 <sup>fe</sup>	9.11 <sup>fe</sup>	10.08 <sup>e</sup>	19.61 <sup>a</sup>	15.50 <sup>c</sup>	13.44 <sup>d</sup>
<b>LSD(0.05)</b>	<b>1.52</b>							
<b>CV (%)</b>	<b>15.82</b>							

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

Table 5.4.  $\text{NO}_3^-$ -N contents ( $\text{mg kg}^{-1}$ ) of soils collected from the Wondo Genet district during different incubation periods in laboratory soil

Dry Azolla rate( $\text{g kg}^{-1}$ )	Incubation periods							
	0	7	14	21	28	42	49	56
0	1.08 <sup>m</sup>	1.09 <sup>m</sup>	1.07 <sup>m</sup>	1.08 <sup>m</sup>	1.08 <sup>m</sup>	1.05 <sup>m</sup>	1.05 <sup>m</sup>	1.07 <sup>m</sup>
15	1.08 <sup>m</sup>	3.35 <sup>l</sup>	3.54 <sup>l</sup>	3.12 <sup>l</sup>	5.51 <sup>jk</sup>	6.46 <sup>jk</sup>	4.25 <sup>l</sup>	3.79 <sup>l</sup>
30	1.09 <sup>m</sup>	9.09 <sup>hi</sup>	10.06 <sup>gh</sup>	10.78 <sup>fg</sup>	12.39 <sup>d</sup>	20.89 <sup>b</sup>	17.71 <sup>c</sup>	12.91 <sup>d</sup>
45	1.07 <sup>m</sup>	10.25 <sup>fg</sup>	11.88 <sup>fe</sup>	13.31 <sup>de</sup>	12.53 <sup>d</sup>	24.26 <sup>a</sup>	20.82 <sup>b</sup>	17.73 <sup>c</sup>
<b>LSD</b>	<b>1.15</b>							
<b>CV (%)</b>	<b>8.16</b>							

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

### 5.3.3. Total mineral N ( $\text{NH}_4^+$ -N + $\text{NO}_3^-$ -N) release

At the Hawassa Zuria and Wondo Genet sites, the combination of Azolla and the incubation days had a substantial impact on the overall availability of N ( $\text{NH}_4^+$ -N +  $\text{NO}_3^-$ -N) ( $p < 0.01$ ; Tables 5.5 and 5.6). Azolla was applied at a rate of 45  $\text{g kg}^{-1}$ , and after 42 days of incubation, the total mineral N concentration at the Hawassa and Wondo Genet locations was 48.57  $\text{mg N kg}^{-1}$  and 39.88  $\text{mg N kg}^{-1}$ , respectively (Tables 5.5 and 5.6).

Furthermore, different incubation periods also had an impact on the mean mineral N ( $p < 0.001$ ; Table 5.5 and 5.6). At 42 days of incubation at both locations, the highest mean mineral N content was 45  $\text{g kg}^{-1}$ , followed by 30  $\text{g kg}^{-1}$ , with the lowest total mineral N found in the control treatments (Table 5.5 and 5.6). At both the Hawassa Zuria and Wondo Genet locations, the total

available N content was significantly affected by the combination of treatments ( $p < 0.01$ ; Table 5.5 and 5.6). at 42 days of incubation at both locations, the highest mean mineral N content was  $45 \text{ g kg}^{-1}$ , followed by  $30 \text{ g kg}^{-1}$ , with the lowest total mineral N found in the control treatments (Table 5.5 and 5.6). The study found significant effects on total mineral N concentration in Hawassa Zuria and Wondo Genet soils treated with  $45 \text{ g kg}^{-1}$  N, with the highest value in the control treatments, and treatment combination and incubation day. The total changes in total inorganic N, ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) in soil treated with Azolla compared to those in the control without amendments are shown in Table 5.5 and 5.6. After adding Azolla, the total amount of inorganic N in the control soil increased significantly ( $p \leq 0.05$ ) to  $16.7 \text{ mg kg}^{-1}$  during incubation. After 42 days of incubation, the maximum mineralization potential of total mineralized N was shown by the  $45 \text{ g kg}^{-1}$  Azolla in Hawassa and Wondo Genet, which released 39.88 and 48.57 mg of mineral N  $\text{kg}^{-1}$  soil, respectively (Table 5.5 and 6.5). The study found that from days 42 to 49, a significant amount of nitrogen was mineralized, with differences between treatments. The total available nitrogen increased with the rate of Azolla in both locations. This indicates the potential for mineralization.

At the end of the 56-day incubation experiment in Hawassa Zuria and Wondo Genet locations, the net mineralization of nitrogen, defined as the sum of the ( $\text{NH}_4^+$ -N treatment +  $\text{NO}_3^-$ -N treatment) – ( $\text{NH}_4^+$ -N control +  $\text{NO}_3^-$ -N control), showed the following results: Azolla ( $45 \text{ g kg}^{-1}$ ) ( $23.49$  and  $33.27 \text{ mg kg}^{-1}$ ) > Azolla ( $30 \text{ g kg}^{-1}$ ) ( $17.06$  and  $23.80 \text{ mg kg}^{-1}$ ) > Azolla ( $15 \text{ g kg}^{-1}$ ) ( $4.01$  and  $5.15 \text{ mg kg}^{-1}$ ) > control ( $0.01$  and  $0.02 \text{ mg kg}^{-1}$ ), respectively. In Wondo Genet locations, the net mineralization of nitrogen was higher than in the Hawassa location. Similar findings were reported by Jama (2018), Sukor (2013), O'hara (2001), and Abbasi and Khaliq (2016). The high rates of Azolla and Azolla + Watanabe resulted in the highest total available N ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) concentrations and N availability compared to the composted manure and control treatments. Calderón et al. (2005) found that the chemical composition of organic matter affects the amount of nitrogen released into crops.

In line with this, studies conducted by Eghball (2000) and Abebe (2018) indicated that nitrogen (N) mineralization increases with rising temperatures in agricultural soils. Schomberg et al. (2009) also confirmed that the mineralization of organic nitrogen sources depends on environmental variables such as water and temperature. Huang et al. (2004) found that manure in soils boosts soil microbial population, consuming more nitrogen than mineralization processes release. This

can reduce nitrogen availability, especially in agroecosystems with limited nitrogen availability. The main source of N for crops and microbes is SOM, via mineralization. High-quality organic matter has a low C:N ratio and sufficient N to sustain microbes and crop growth (Ross et al., 2009). Nitrogen mineralization differed among the organic amendments, with the highest N mineralization in amended soil (Masunga et al., 2016).

Table 5.5. Total mineral nitrogen ( $\text{NH}_4^+ + \text{NO}_3^-$ ) concentration (mg/kg soil) of the treatments for the various incubation periods (days) of the soil collected from the Hawassa Zuria district

Dry Azolla rate(g kg <sup>-1</sup> )	Incubation periods							
	0	7	14	21	28	42	49	56
Control	1.33 <sup>n</sup>	1.33 <sup>n</sup>	1.34 <sup>n</sup>	1.33 <sup>n</sup>	1.33 <sup>n</sup>	1.32 <sup>n</sup>	1.33 <sup>n</sup>	1.36 <sup>n</sup>
15	1.33 <sup>n</sup>	2.50 <sup>n</sup>	4.91 <sup>m</sup>	5.28 <sup>m</sup>	7.48 <sup>lk</sup>	8.55 <sup>k</sup>	6.63 <sup>lkm</sup>	5.40 <sup>lk</sup>
30	1.33 <sup>n</sup>	11.55 <sup>j</sup>	12.19 <sup>j</sup>	14.51 <sup>ih</sup>	15.67 <sup>gh</sup>	34.71 <sup>b</sup>	27.36 <sup>d</sup>	18.45 <sup>f</sup>
45	1.33 <sup>n</sup>	13.23 <sup>ji</sup>	16.42 <sup>gfh</sup>	16.87 <sup>gf</sup>	18.23 <sup>f</sup>	39.88 <sup>a</sup>	29.91 <sup>c</sup>	24.88 <sup>e</sup>
<b>LSD(0.05)</b>	<b>2.19</b>							
<b>CV (%)</b>	<b>12.39</b>							

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

Table 5.6. Total mineral nitrogen ( $\text{NH}_4^+ + \text{NO}_3^-$ ) concentration (mg/kg soil) of the treatments for the various incubation periods (days) of the soil collected from the Wondo Genet Zuria district

Dry Azolla rate(g kg <sup>-1</sup> )	Incubation periods							
	0	7	14	21	28	42	49	56
Control	1.94 <sup>o</sup>	1.94 <sup>o</sup>	1.93 <sup>o</sup>	1.96 <sup>o</sup>	1.94 <sup>o</sup>	1.93 <sup>o</sup>	1.94 <sup>o</sup>	1.96 <sup>o</sup>

15	1.93 <sup>o</sup>	5.99 <sup>n</sup>	6.18 <sup>n</sup>	5.85 <sup>n</sup>	9.04 <sup>m</sup>	11.07 <sup>l</sup>	7.97 <sup>mn</sup>	7.13 <sup>n</sup>
30	1.94 <sup>o</sup>	16.09 <sup>k</sup>	17.40 <sup>ijk</sup>	19.00 <sup>hi</sup>	21.52 <sup>g</sup>	42.24 <sup>b</sup>	35.67 <sup>c</sup>	25.78 <sup>d</sup>
45	1.93 <sup>o</sup>	18.02 <sup>ij</sup>	20.73 <sup>hg</sup>	24.34 <sup>de</sup>	23.31 <sup>ef</sup>	48.57 <sup>a</sup>	41.11 <sup>b</sup>	35.25 <sup>c</sup>
<b>LSD(0.05)</b>	<b>1.78</b>							
<b>CV (%)</b>	<b>6.85</b>							

Means values followed by the same letter (s) are not significantly different from each other at the 5% level of significance.

#### 5.3.4. Soil N mineralization rate under end of incubation periods

Soil net N mineralization rates were calculated by subtracting the final (day 56) soil inorganic N from the initial (day 0) soil inorganic N and dividing the resulting number by the number of incubation days (Jama, 2018). At the end of the 56-day incubation period, soil nitrogen mineralization rates in both locations showed a pattern of increasing with a higher rate of Azolla application, followed by a gradual increase in the soil nitrogen mineralization rates during the incubation process. A peak was observed with the 45 g kg<sup>-1</sup> Azolla treatment when compared to the 30 g kg<sup>-1</sup>, 15 g kg<sup>-1</sup>, and control treatments in both locations (Figure 5.1). Initially, the differences in soil nitrogen mineralization among the fertilization treatments were minimal, but these differences became more significant as the incubation progressed. The highest rate of soil nitrogen mineralization was with the 45 g kg<sup>-1</sup> Azolla treatment (0.421 and 0.595 mg N kg<sup>-1</sup> day<sup>-1</sup>) on day 56 in the Hawassa and Wondo Genet districts, respectively (Figure 5.1). The lowest rate of nitrogen mineralization was recorded in the control group (0.0141 and 0.099 mg N kg<sup>-1</sup> day<sup>-1</sup>) in both districts, respectively (Figure 5.1). The soils collected from the Hawassa Zuria and Wondo Genet locations showed that at the end of incubation, the accumulated mineralization of inorganic nitrogen rate in the treatments with the application of Azolla was in the order of control < 15 < 30 < 45 g kg<sup>-1</sup> (Figure 5.1). At the end of the incubation period, nitrogen mineralization rates were much higher in Wondo Genet than in Hawassa Zuria locations (Figure 5.1).

In the current study, we found that the interaction between the rate of Azolla and the incubation time had a significant impact on the soil nitrogen mineralization rate at the end of the 56-day incubation period. The fastest rate of soil nitrogen mineralization was observed at 45 g kg<sup>-1</sup> of Azolla (Figure 5.1). A similar trend was observed for treatments with inorganic nitrogen at the

end of the incubation experiment. The statistical results indicated that the Hawassa Zuria district had less accumulated mineralized nitrogen than the Wondo Genet district, which may be due to the higher pH of the soil in the Hawassa Zuria district (Figure 5.1). These findings are consistent with the results reported by Pathak and Rao (1998), who noted that total inorganic N decreased in soils with high pH during incubation. The values of various forms of nitrogen in the inorganic treatment group were higher compared to the organic and control groups. This increase may be attributed to the high nitrogen content resulting from the addition of urea, which dissolves in water and is quickly converted to ammonia (Ma et al., 2019). Huang et al. (2004) found that manure in soils boosts soil microbial population, consuming more nitrogen than mineralization processes release. This can reduce nitrogen availability, especially in agroecosystems with limited nitrogen availability.

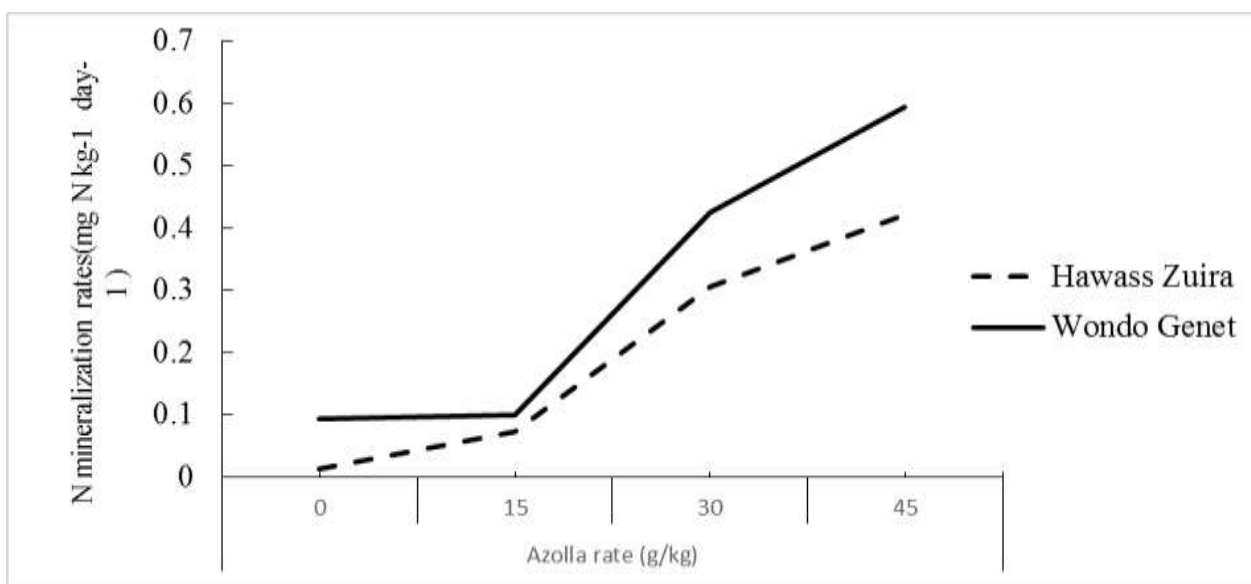


Figure 5.1. Soil N mineralization rates ( $\text{mg N kg}^{-1} \text{ day}^{-1}$ ) among Azolla as a function of time during the 56-day incubation study.

### **5.3.5. Changes in the organic carbon content, total nitrogen content, and C: N ratio in Azolla**

#### **5.3.5.1. Release of organic carbon**

The patterns of organic carbon (OC) release from the treatments with Azolla are presented in Figure 2a, b for Hawassa Zuria and Wondo Genet locations, respectively. It was observed that the 45 g kg<sup>-1</sup> treatment resulted in the highest release of OC (3.21 and 4.53%) on day 14 of incubation at Hawassa Zuria and Wondo Genet districts, respectively (Figure 5.2a, and b). This is due to the soil in the Hawassa Zuria and Wondo Genet districts being characterized as sandy loam and sandy clay loam, respectively. Generally, the soils from Hawassa Zuria released less OC compared to those from Wondo Genet. The OC content in Wondo Genet was higher than in Hawassa Zuria, which could be due to the nature of the soil. Throughout the incubation periods, significant differences in organic carbon were noted among the Azolla treatments, particularly between days 42 and 49, after which a decline in organic carbon levels was recorded until day 56 during mineralization (Figure 5.2a and b).

We found that the rate of Azolla biomass production and incubation time had the most significant impact on the release of organic carbon. Organic carbon release increased significantly in both locations as the amount of Azolla biomass increased (Figure 5.2a and b). Organic carbon (OC) was highest in the 45 g kg<sup>-1</sup> Azolla treatment, as predicted, followed by 30 g kg<sup>-1</sup> Azolla treatment, and finally the 15 g kg<sup>-1</sup> Azolla treatment at 14 days of incubation in both locations (Figure 5.2 a and b). Similar findings were reported by Kuśmierz et al. (2023), soil texture is considered an important factor influencing soil organic carbon accumulation. High clay content has a greater potential for storing organic carbon compared to sandy soil (Wiesmeier et al., 2019). This is because sandy soil, with its loose structure, large sand particles, small surface area, and high water permeability, makes it easy for organic carbon to be decomposed or lost by microorganisms. According to Setiawati et al. (2018), who was reported that a mixture of 50% Azolla and 50% manure can increase the organic carbon content of soil from 1.3% to 1.7%. This suggests that restoring unhealthy soil can lead to the development of healthy soil. The use of narrow C/N ratio materials like Azolla enhances soil organic carbon and other nutrient inputs while minimizing N losses. Yadav et al., 2020; Bhardwaj et al., 2023) and may be an appropriate option for reducing methane emissions (Korsa et al., 2024). Mineralization tends to be higher in coarse-textured soil and decreases as

the clay content increases (Deenik, 2006). Furthermore, organic matter content might influence microbial community structure directly by providing a favorable habitat for specific bacteria, hence maximizing the degradation process (Girvan et al., 2003). Low nitrogen retention has been seen in sand and fine loamy soil. Clay soil retains nitrogen well because of its high water-holding capacity, nutritional content, and soil microbial life. This results in high nitrogen retention during the mineralization process. Generally, the soils from Hawassa Zuria released less OC compared to those from Wondo Genet. The OC content in Wondo Genet was higher than in Hawassa Zuria, which could be due to the nature of the soil. Throughout the incubation periods, significant differences in organic carbon were noted among the Azolla treatments, particularly between days 7 and 14, after which a decline in organic carbon levels was recorded until day 56 during mineralization.

Similar findings were reported by Jama et al. (2018), indicating that the addition of Azolla fertilizers enhances soil fertility and organic carbon levels. Azolla is rich in nitrogen and features a low carbon-to-nitrogen (C/N) ratio, making it effective in sustaining microbial biomass and promoting crop growth. In vegetable crops, the C ratio of Azolla + Watanabe fertilizer was lower than that of combined Azolla and compost fertilizers. The chemical composition of organic matter dictates the nitrogen availability for crops (Calderón et al., 2005). Azolla enriches the soil with organic matter, supporting nutrient availability for both current and future crops (Oyange et al., 2020). Azolla compost improves organic carbon levels, which enhance microbial growth and can boost tomato yields by 20-30% (Sarolkar, 2022). Its low cost and eco-friendly nature make it a viable direct fertilizer option for tomatoes. The current study demonstrated that Azolla significantly affects carbon release, improving nitrogen accessibility in the soil. Organic carbon levels varied between treatments, influenced by the rate of Azolla applied and the incubation period. Organic carbon and total nitrogen are critical indicators of soil fertility and quality, closely linked to productivity in agricultural systems (Li et al., 2022). Additionally, Azolla is effective in fixing atmospheric nitrogen through its symbiotic relationship with cyanobacteria (Marzouk et al., 2024). Halber and Kheroar (2013) found that the addition of Azolla and cyanobacteria increases the amount of organic carbon after a specific period of mineralization. Studies have shown that incorporating organic additions, like Azolla compost, into rice fields can elevate levels of organic carbon (OC) (Novair et al., 2020).

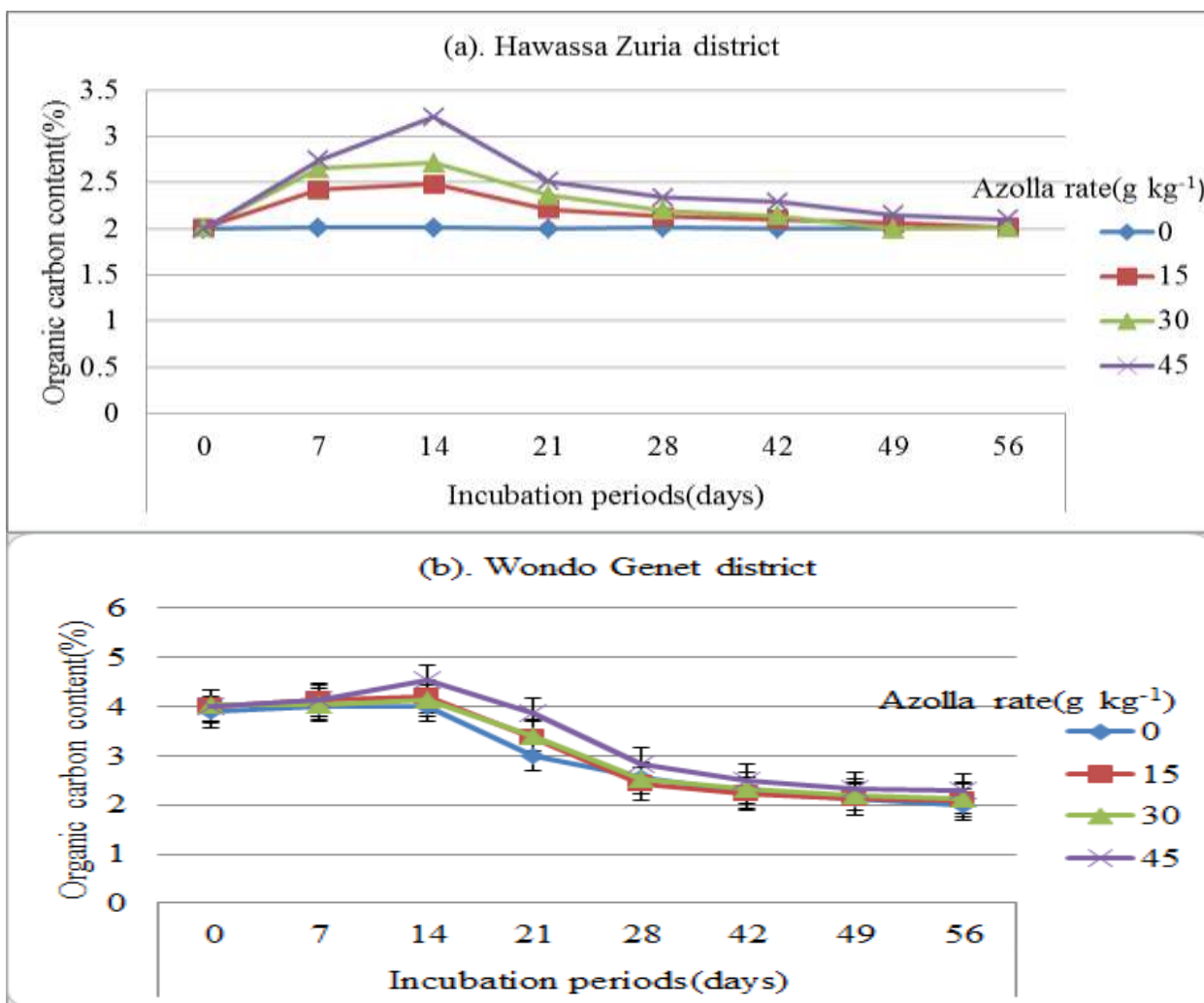


Figure 5.2. The effects of Azolla rate on organic carbon content at different incubation periods at Wondo Genet soil the (a) Hawassa Zuria and (b) Wondo Genet districts.

### 5.3.5.2. Release of total nitrogen

Total nitrogen was significantly ( $P < 0.001$ ) affected by the interaction effects of the Azolla rate and incubation periods. The total nitrogen content release patterns for the four Azolla treatments at varying incubation durations are shown in the Hawassa and Wondo Genet sites, respectively (Figure 5.3a and b). In both sites, the total nitrogen released generally increased between 0 and 14 days and then decreased from 21 days of incubation for all treatments (Figure 5.3a and b). From day 28 to day 56 of incubation, the total nitrogen content was released in a low pattern. A high amount of nitrogen was released overall between days 7 and 14 (Figure 5.3a and b). It was observed that the treatment with  $45 \text{ g kg}^{-1}$  resulted in the highest release of TN (0.25% and 0.44%) on day 14 of incubation in the Hawassa Zuria and Wondo Genet districts, respectively (Figure 5.3a and b). Generally, the soils from Hawassa Zuria

released less TN compared to those from Wondo Genet. Throughout the incubation periods, significant differences in total nitrogen were noted among the Azolla treatments, particularly between days 7 and 14, after which a decline in nitrogen and carbon levels was recorded until day 56. This is because in the mineralization process, the overall process eventually leads to improved soil fertility and potentially long-term carbon stabilization in the form of humic substances, the initial phase involves a net loss of carbon from the total mass as it is released as gas (Ebrahim et al., 2024). Laboratory analyses indicated that the 30 and 45 g kg<sup>-1</sup> Azolla treatments had the highest total nitrogen levels during mineralization.

In both locations, the total nitrogen release pattern of different Azolla biomass rates at different incubation periods (days) was similar, with the first two weeks (7-14 days) releasing more TN than the 21-56 days of incubation (Figure 5.3a and b). Supporting this, research by Fu-sheng et al. (2007) indicated that nitrogen mineralization is influenced by soil total N, the addition of organic fertilizers, and environmental factors. According to Lestari et al. (2024), the high nitrogen concentration shows that Azolla species has the natural ability to fix nitrogen in the air. This is in line with (Yao et al., 2018) groundbreaking research on Azolla's nitrogen-fixing abilities. Conversely, the dehydration procedure had a significant impact on nitrogen levels in dried Azolla, resulting in a decrease of 3%. According to Syamsiyah et al. (2017), using Azolla enhanced soil total nitrogen by 16% when compared to the control. According to Chang et al. (2023), Azolla's high nitrogen content and low C/N ratio can improve soil ammonium and nitrification, increase inorganic nitrogen levels in the soil, and promote microorganism growth. The experiments reported here, as well as previous ones (Ventura et al., 1992), confirmed that Azolla N mineralization and its availability to rice are largely determined by the N contents in Azolla. According to Asuming-Brempong et al. (2008) when within 40–50 days after incorporating fresh azolla in paddy fields, approximately about 60% of its organic nitrogen had been mineralized. After that period, nitrogen mineralization was low.

Similar findings were reported by Jama (2018), indicating that the addition of Azolla fertilizers enhances soil fertility and organic matter levels. Azolla is rich in nitrogen and features a low C/N, making it effective in sustaining microbial biomass and promoting crop growth. According to Asuming-Brempong et al. (2008), the total nitrogen mineralized from fresh Azolla was high starting on day 40, with the highest amount occurring on day 50, where

nitrogen mineralized was significantly different from the control. However, on day 60, the total nitrogen mineralized began to decline with the fresh Azolla treatment. A similar report was found by Setiawati et al. (2018), indicating that during mineralization, organic nitrogen is rapidly mineralized from fresh Azolla during the first two weeks and then at a more gradual rate. Azolla enriches the soil with organic matter, supporting nutrient availability for both current and future crops (Oyange et al., 2020). The current study demonstrated that Azolla significantly affects nitrogen and carbon release, improving nitrogen accessibility in the soil. Total nitrogen is a critical indicator of soil fertility and quality, closely linked to productivity in agricultural systems (Li et al., 2022). Additionally, Azolla is effective in fixing atmospheric nitrogen through its symbiotic relationship with cyanobacteria (Marzouk et al., 2024).

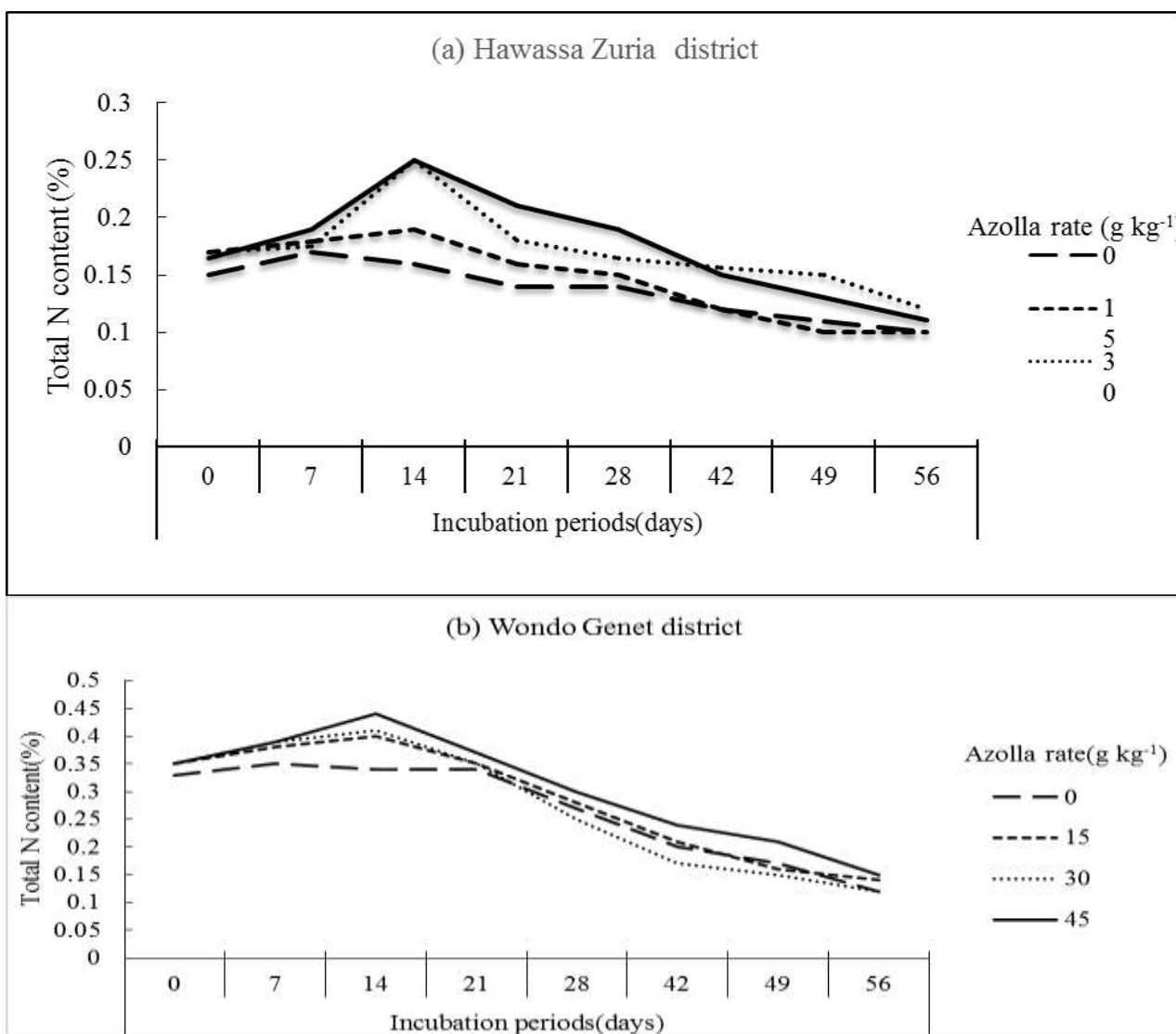


Figure 5.3. The effects of Azolla rate on released total nitrogen content at different incubation periods at Wondo Genet soil the (a) Hawassa Zuria and (b) Wondo Genet districts.

### 5.3.5.3. Organic carbon to total nitrogen ratio of Azolla

Soils collected from locations treated with 45 or 30 g kg<sup>-1</sup> released the most NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N due to the relatively high levels of Azolla. In both soil locations, it is preferred to apply Azolla biomass with a low C/N ratio and adequate N to support crop growth and microbial biomass (Figures 5.4a and b). A favorable environment for urease activity promotes rapid hydrolysis of urea fertilizer and the breakdown of Azolla, leading to increased levels of NH<sub>4</sub><sup>+</sup>-N in all situations (Jahangir et al., 2021). Despite the higher N content in the Azolla, the plants in the control treatment had the lowest levels of mineral NH<sub>4</sub><sup>+</sup>-N. N-rich such as Azolla have potential as nitrogen fertilizers with delayed release (Jama et al., 2023). According to Setiawati et al. (2018), the carbon-to-nitrogen (C/N) ratio of Azolla significantly impacts the balance of mineralization and immobilization. Azolla is a common organic fertilizer in rice cropping systems; however, rice straw takes longer to mineralize due to its higher C/N ratio (Hung et al. 2016). VanHung et al. (2020) believe that Azolla can help alleviate this condition. Azolla is an eco-friendly amendment that also enhances nitrogen use efficiency (Cissé and Vlek, 2022). According to Jama et al. (2023), who found that the higher C/N ratios of fertilizers or soil amendments (particularly over 18 C/1N) tend to result in higher immobilization of N and a slower release of inorganic N for plant uptake, Azolla + Watanabe fertilizer had a lower C/N ratio than Azolla and compost fertilizers.

Nitrogen content, C/N ratio, lignin, cellulose and hemicellulose contents, and polyphenols can all influence how much nitrogen is released in to soils (Mohanty et al., 2011). Similarly, Singh (1981), Bhuvaneshwari (2012), and Bhuvaneshwari and Kumar (2013) found a significantly high mean nitrogen value due to Azolla's high nitrogen content. The accumulation of NO<sub>3</sub><sup>-</sup>-N was significantly greater in the 45 g kg<sup>-1</sup> treatment than in the other treatments due to higher NH<sub>4</sub><sup>+</sup>-N production, which led to rapid nitrification. Azolla with high nitrogen contents and low C/N ratios provide more mineral N through mineralization (Cordovil et al., 2005). The total nitrogen concentration remained higher than that in the initial unamended soil after 56 days. A lower C/N ratio in response to soil amendments enhanced mineralization, resulting in a decrease in the total nitrogen concentration after 56 days compared to the initial total nitrogen (TN) concentration, consistent with the findings of other authors (Uddin et al., 2021). The significant decreases in the TN and OC contents varied on each sampling day from 21 to 56 days, showing similar trends at both locations. Consequently, the C/N ratio decreased

during mineralization. Perez-Harguindeguy et al. (2000) reported that the C/N ratio was a good indicator of the mineralization rate, with higher C/N values often associated with compounds exhibiting greater C enrichment.

At Wondo Genet, mineralization occurred over an incubation period of 49-56 days with a wide C/N ratio (12-18:1), followed by 42 days (10-14:1) and 0-28 days (9-12:1), indicating a narrowing C/N ratio (Figure 4b). Mary et al. (1996) confirmed that organic residues with low C/N ratios exhibit more mineralization of N, while wide C/N ratios may cause immobilization of N. Hawassa Zuria has a higher C/N ratio during mineralization than Wondo Genet does, possibly due to differences in precipitation, soil properties, and microbial factors. A higher C/N ratio of fertilizer or soil amendment (especially above 18 C:1 N) can lead to greater immobilization of N and slower inorganic N release for plant uptake (Jama et al., 2023). At the end of the 56-day incubation period, treatments with slower mineralization of N and less availability of N were observed in the control (C/N = 20:1, 17:1), 15 g kg<sup>-1</sup> (C/N = 20:1, 13:1), 30 g kg<sup>-1</sup> (C/N = 17:1, 18:1), and 45 g kg<sup>-1</sup> (C/N = 19:1, 15:1) treatments at both the Hawassa Zuria and Wondo Genet sites (Figure 5.4a and b). After 56 days of incubation under laboratory conditions, the net available N fell exponentially in proportion to the C/N ratio, demonstrating that greater C/N ratios result in lower N availability in the soil and vice versa. Different authors have reported similar findings, showing that higher C/N ratios lead to lower N availability in the soil (Islam et al., 2021). The maximum net mineralization of N in Azolla-treated soils is frequently attributed to additions with a low C/N ratio and a high N content (Flavel et al., 2006).

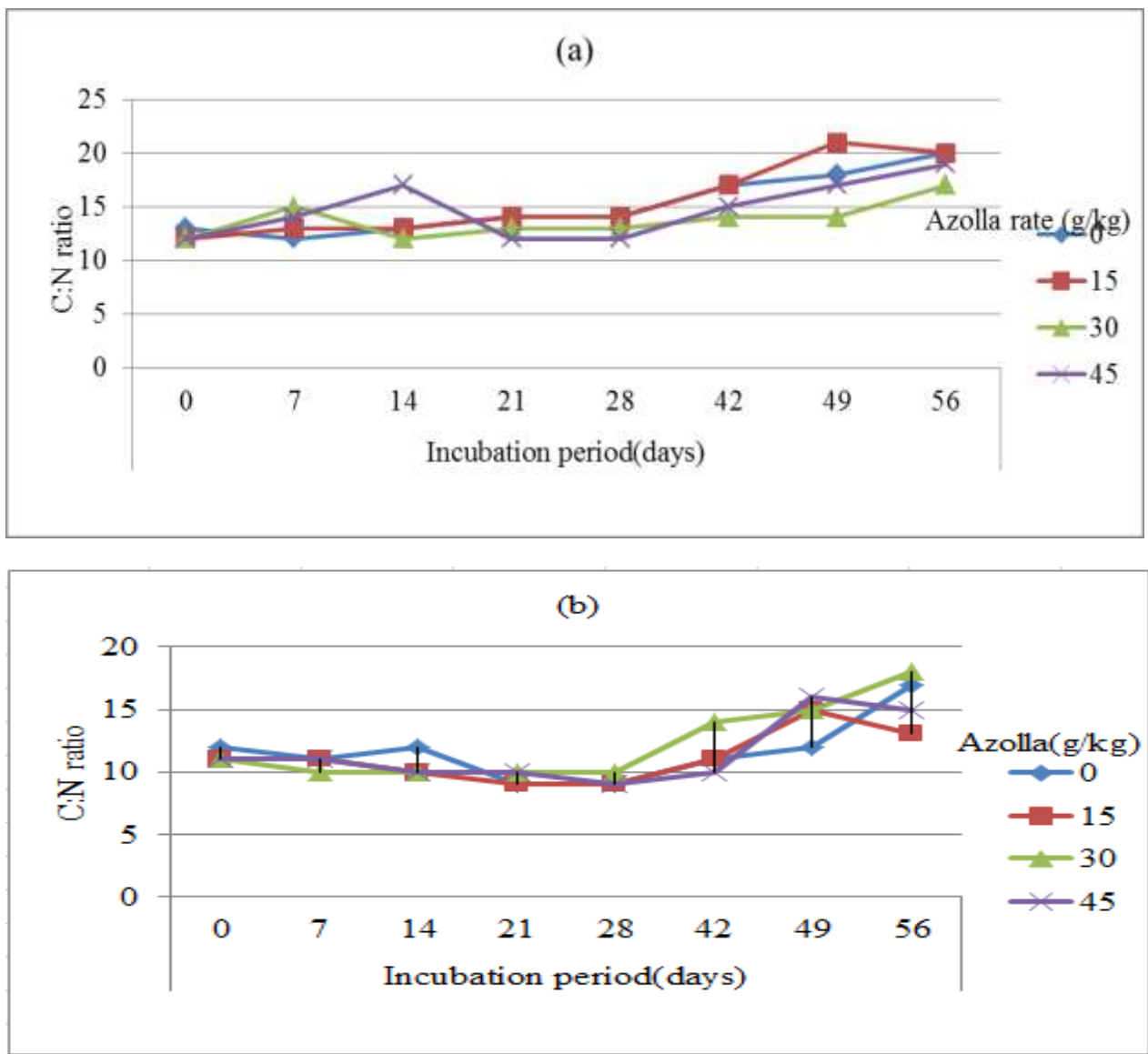


Figure 5.4. Carbon-to-nitrogen ratio (C/N) of Azolla after 0-56 days of incubation in the a) Hawassa Zuria and b) Wondo Genet districts.

#### 5.4. Conclusions

The soil collected from Hawassa Zuria and Wondo Genet districts, the maximum amounts of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ -N occurred at 42 days of incubation with an Azolla dry biomass rate of 45 g  $\text{kg}^{-1}$  (20.27 and 24.31 mg  $\text{kg}^{-1}$ ) and  $\text{NO}_3^-$ -N (19.61 and 24.26 mg  $\text{kg}^{-1}$ ), respectively. Similarly, the total mineralized N in the Azolla dry biomass treatment (45 g  $\text{kg}^{-1}$ ) increased during the 42-day incubation period compared to other treatments. In both locations, the concentrations of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N gradually increased in soils amended with different Azolla from 0 to 42 days of incubation, then declined until 56 days of incubation. The highest nitrogen mineralization occurred after 42 days of incubation with 45 g  $\text{kg}^{-1}$  of Azolla in soils from both

locations (Hawassa Zuria and Wondo Genet). After 42 days of incubation, Azolla dry biomass showed the highest total mineralized N levels (39.88 and 48.57 mg kg<sup>-1</sup> soil), while the control treatment had the lowest levels, possibly due to its high C/N ratio. Although the biomass of Azolla used varied, the mineralization of N in the other treatments changed over the course of several days of incubation. All the modified treatments produced significantly greater total N mineralization than the controls at both locations. Naturally, fertile soils have a high potential for nitrogen mineralization. According to the present study, the rate at which mineral nitrogen is increased by Azolla increases, as does the duration of nitrogen availability. Additionally, the total N and C contents of Azolla are also important factors in its N-release capacity. In future research, these Azolla should be tested in a field experiment to confirm the results of the laboratory incubation experiment.

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## CHAPTER SIX

### 6. GENERAL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Declining soil fertility is a serious limitation to crop production in Ethiopia. The primary causes are loss of organic matter, soil nutrient depletion, acidity, salinity, topsoil erosion, and deterioration of physical soil properties. The present study aimed at enhancing the soil fertility and productivity of tomatoes through the integrated application of Azolla and inorganic N fertilizers.

A total of four studies were conducted in two districts of the Sidama region of southern Ethiopia. The first study aimed to characterize and classify the soils of the Jara Gelalicha and Aruma areas of the Sidama region, southern Ethiopia. For each site, one representative soil pedon was excavated, described, and classified based on morphological and physicochemical properties of the soils. A total of 12 disturbed and 12 undisturbed soil samples were collected from each diagnostic horizon of both locations. The results showed that the surface horizon textural of the pedon of Jara Gelalicha was sandy loam, whereas the textural class of the pedon at Aruma was sandy clay loam. In the Jara Gelalicha and Aruma locations, the organic carbon content in the surface horizons was 2.39% and 3.57%, respectively. The total nitrogen content of the surface soil horizons in Jara Gelalicha and Aruma locations was 0.23% and 0.32%, respectively. Based on the World Reference Base for Soil Resources, the soils of the study areas were classified as Eutric Cambisols (Loamic) in Jara Gelalicha and Eutric Andosols (Loamic) in Aruma. Therefore, the soil fertility management and land use decisions should consider such soil variability. The second study was titled “Influence of Azolla and inorganic nitrogen fertilizers on soil chemical properties, yield, and yield components of tomato under greenhouse conditions.” The aim of this study was to investigate the impacts of Azolla dry biomass and inorganic N-fertilizer applications on soil chemical properties soil samples were collected at a depth of 0-20 cm from the Hawassa Zuria and Wondo Genet locations as well as to determine the optimum combination of Azolla dry biomass and N-fertilizer for maximizing tomato yield. The study used a factorial combination of four rates of Azolla dry biomass (0, 25, 50, and 75 g pot<sup>-1</sup>) and four levels of inorganic N (0, 0.23, 0.46, and 0.69 g pot<sup>-1</sup>) in a completely randomized design with three replications. The results showed that in both locations, the combined application of Azolla as a source of organic N and inorganic N

fertilizers improved chemical properties of soil, promoted tomato growth, and increased tomato productivity. The combined application of Azolla dry biomass and inorganic nitrogen offers a viable approach to enhance tomato productivity and soil fertility. In both soil collected locations, the combined application of 75 g Azolla pot<sup>-1</sup> and 0.69 g N pot<sup>-1</sup> resulted in the greatest improvements in critical parameters, including plant height, number of branches per plant, fruit diameter, number of fruits per plant, and number of clusters per plant. The highest marketable fruit yields in the soil collected from Hawassa Zuria and Wondo Genet were 1088.43 g/plant and 833.73 g/plant, respectively. The integrated application of Azolla and inorganic N offers a viable approach to enhance tomato productivity and soil fertility. It can be concluded that an optimum combination of Azolla and inorganic N fertilizers remains essential for optimal tomato growth and yield, emphasizing that the integrated application of organic and inorganic fertilizers is the best method of farming practice. The third study was titled “Enhancing soil fertility, crop productivity and nitrogen use efficiency through integrated use of Azolla dry biomass and nitrogen fertilizers under irrigated Conditions.” The aim of this study to determine the effects of Azolla dry biomass as an organic nitrogen source on soil chemical properties and tomato production, determine the interaction effects of Azolla dry biomass and inorganic nitrogen on tomato yield and yield components, N uptake, and N use efficiency in tomato production, and as well as to identify the optimum combination of Azolla dry biomass and N fertilizers for maximizing tomato productivity. The experiment tested four levels of Azolla dry biomass (0, 5, 10, and 15 t ha<sup>-1</sup>) and four levels of inorganic nitrogen (0, 46, 92, and 138 kg N ha<sup>-1</sup>) arranged in a factorial combination using a randomized complete block design (RCBD) with three replications. The combined two-year data showed that the highest marketable fruit yields in the Hawassa Zuria and Wondo Genet locations were 23.76 t ha<sup>-1</sup> and 26.35 t ha<sup>-1</sup>, respectively. This was achieved by adding 15 t ha<sup>-1</sup> of Azolla biomass and 138 kg ha<sup>-1</sup> of nitrogen. The highest agronomic efficiency of N (142.39 kg kg<sup>-1</sup> and 139.35 kg kg<sup>-1</sup>) was obtained with the combined application of 0 t ha<sup>-1</sup> of Azolla and 46 kg N ha<sup>-1</sup> in Hawassa Zuria and Wondo Genet, respectively, while the control had the lowest (4.91 kg kg<sup>-1</sup> and 3.48 kg kg<sup>-1</sup>, respectively). In conclusion, tomato has significantly responded to the application of N fertilizer, Azolla, and their combined application relative to the control in both locations, indicating that the soil N content in both locations is insufficient for optimum production of tomato. Significantly, the highest yield of tomato was obtained from treatments involving the integrated application of 15 t ha<sup>-1</sup> and 138 kg N ha<sup>-1</sup> compared

to that obtained from the single application of N fertilizer and Azolla, suggesting that there was a synergistic interaction between the two sources in increasing the growth and yield of tomato. The fourth study was “Effects of Azolla on soil nitrogen mineralization in soils collected from Hawassa Zuria and Wondo Genet districts incubated under laboratory conditions.” The aim of this experiment was to determine the effects of Azolla dry biomass rates and incubation periods on soil nitrogen mineralization under laboratory settings. The experiment was conducted in the soil laboratory at College of Agriculture, Hawassa University. Soil samples were collected from Hawassa Zuria and Wondo Genet districts and incubated for 56 days at dark 25°C with in plastic jar. Four levels of Azolla (0, 15, 30, and 45 g kg<sup>-1</sup> of soil) and eight incubation durations (0, 7, 14, 21, 28, 42, 49, and 56 days) were arranged in a factorial order and laid out in a completely randomized design (CRD) with three replications. The results show that Azolla amendments increased both the rate and total amount of nitrogen mineralized in the soil, with the highest mineralization observed at a 45 g kg<sup>-1</sup> Azolla rate after 42 days of incubation. Specifically, the total nitrogen mineralized (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) in soils from Hawassa and Wondo Genet reached 39.88 mg kg<sup>-1</sup> and 48.57 mg kg<sup>-1</sup>, respectively, under this treatment. The concentrations of both NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N rose gradually between day 0 and day 42, but then began to fall. The concentrations of both NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in the Hawassa Zuria and Wondo Genet locations decreased at the end of the incubation period. Mineralization rates peaked on day 42, at which time the highest amounts of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were frequently recorded 42 days after the application of Azolla.

Based on the current study, the following key recommendations have been developed.

- Site-specific soil characterization can be an effective method of soil characterization and easily understand the physicochemical properties of soil. Their activities should be considered in site-specific soil classification and management. Moreover, a morphological and physicochemical soil properties examination indication is recommended to support this finding.
- The combined application of Azolla and N fertilizers should improve both the yield of tomato production and improve soil fertility. For optimal yield and increased productivity of tomatoes, it is recommended to use a combination of 15 t ha<sup>-1</sup> of Azolla and 138 kg ha<sup>-1</sup> of inorganic N fertilizers in the studied areas and other locations with similar agroecological conditions.

- The maximum amount of inorganic nitrogen ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) was observed under laboratory conditions for 42 days with  $45 \text{ g kg}^{-1}$  Azolla. However, further testing under actual field conditions is needed to confirm these findings and determine the maximum levels of mineral nitrogen.

## 7. APPENDICES

Appendix Table 2.1: Jara Gelalicha (Hawassa Zuria) Profile description Sheet

Profile No. 1		
Soil Classification	Eutric Cambisols	
Date of examination	December 1, 2022	
Author of description	Habtam Alemayehu	
Location	Jara Gelalicha Kebele, Hawassa Zuria district, Sidama Region	
Coordinate	7°0'29.2''N and 38°23' 35.9''E	
Altitude	1701 meters above sea level	
Surrounding landform	Level land	
Physiographic position	Bottom flat	
Slope (%)	0.2	
Moisture condition	Moist	
Surface drainage	High	
Parent material	Alluvial	
Erosion status	Slightly sheet of erosion	
Present land use type:	Annual field cropping	
Depth (cm)	Horizon	Description
0-18	Ap	Dark gray (5YR4/1) color in moist soil, reddish gray (2.5 YR6/1) in dry soil; moderate, fine and granular; slightly hard, friable; slightly sticky and slightly plastic, sandy clay loam; clear and smooth boundary
18-40	AB	Reddish brown (5YR4/3) in moist soil, light reddish gray (2.5 YR7/1) in dry soil; moderate, medium, angular blocky; lightly hard, friable; slightly sticky, slightly plastic; sandy clay loam; clear and smooth boundary
40-80	Bw	Reddish brown (2.5YR5/4) in moist soil, white(7.5YR8/1) in dry soil; moderate, medium, angular blocky; slightly hard, very firm, slightly sticky, slightly plastic; sandy clay loam; diffuse and smooth boundary
80-110	BC1	Reddish brown (5YR3/4) in moist soil, gray light reddish brown (2.5YR7/1) in dry soil; weak, medium, angular blocky; slightly hard, very firm, slightly sticky, slightly plastic; sandy loam; diffuse and smooth boundary
110-160	BC2	Brown horizon, brown(7.5YR5/4) in moist soil, light gray(10YR7/1) in dry soil; weak, medium, single grain; slightly hard, extremely firm, slightly sticky, slightly plastic; sandy loam; clear and wavy boundary
160-200 <sup>+</sup>	C	Light brown (7.5YR6/3) in moist soil, light reddish brown (2.5Y6/4) in dry soil; weak, medium, massive; slightly hard, extremely firm, no slightly sticky, no plastic; sandy clay loam

Appendix Table 2.2: Aruma (Wondo Genet) Profile description Sheet

Profile No. 1		
Soil Classification	Eutric Andosols	
Date of examination	December 5, 2022	
Author of description	Habtamu Alemayehu	
Location	Aruma Kebele, Wondo Genet district, Sidama Region	
Coordinate	6°58'2.8''N latitude and 38°33'55.5''E longitude	
Altitude	1756 meters above sea level	
Surrounding landform	Plain	
Physiographic position	Upper	
Slope (%)	0.2	
Moisture condition	Moist	
Surface drainage	High	
Parent material	Volcanic ash	
Erosion status	Slightly sheet erosion	
Present land use type:	Annual field cropping	
Depth (cm)	Horizon	Description
0-20	Ap	Very dusky red(2.5YR2.5/2) in moist soil, very dark brown (7.5YR2.5/4) in dry soil;weak,medium and granular;slightly hard, friable; sticky and plastic,sandy clay loam; clear and smooth boundary
20-70	AB	Dark brown (7.5YR3/2) in moist soil to no change in dry soil;weak, medium, angular blocky; hard, friable; very sticky, very plastic; clay loam; diffuse and smooth boundary
70-115	B1	Very dark gray(7.5YR3/1)in moist soil, dark gray(7.5YR4/1) in dry soil; weak, medium, sub angular blocky; hard, firm,slightly very sticky,very plastic;sandy clay loam; diffuse and smooth boundary
115-140	B2	Dark brown (7.5YR3/4)in moist, gray(7.5YR5/2) in dry soil; weak, medium, columnar;very hard, very firable,very sticky,very plastic;sandy clay loam; abrupt and smooth boundary
140-164	2BC	Brown(7.5YR4/4) in moist soil change in to gray(7.5YR5/2) in dry soil; weak, fine, single grain;very hard, very firable,slightly sticky,slightly plastic;sandy clay loam; clear and smooth boundary
164-200 <sup>+</sup>	2C	Brown (7.5YR4/4) in moist soil, no change in dry soil ; weak, fine, single grain;soft, loose,no- sticky,no-plastic;sandy clay loam

Appendix Table 3. 1:ANOVA mean squares of soil chemical properties parameters influenced by Azolla and inorganic N fertilizer application the soil collected from Hawassa Zuria and Wondo Genet districts

Source of Variation	DF	Soil collected from Hawassa Zuria district						Soil collected from Wondo Genet district					
		Mean of squire						Mean of squire					
		Ph	EC	OC	TN	Ava.P	CEC	pH	EC	OC	TN	Ava.P	CEC
Az	3	3.198**	0.28*	2.76**	0.027**	66.06**	52.55**	0.12**	0.086 <sup>ns</sup>	1.99**	5.78**	53.61**	62.87**
N	3	0.007 <sup>ns</sup>	0.007 <sup>ns</sup>	0.60*	0.0097**	11.53 <sup>ns</sup>	5.75 <sup>ns</sup>	0.012 <sup>ns</sup>	0.089 <sup>ns</sup>	0.47**	6.05**	7.24**	7.14 <sup>ns</sup>
Az*N	9	0.019*	0.08 <sup>ns</sup>	0.38**	0.0032**	7.61**	5.23**	0.049 <sup>ns</sup>	0.052 <sup>ns</sup>	0.100**	2.04**	1.40**	18.43**
Error	32	0.0042	0.049	0.21	0.0006	0.47	0.88	0.02	1.89	0.016	2.45	0.82	1.89

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , Az: Azolla; N: nitrogen; EC: electrical conductivity; OC: organic carbon: Ava.P: available phosphorus:CEC: cation exchange capacity.

Appendix Table 3. 2: Mean squares of tomato growth parameters influenced by Azolla bry biomass and inorganic N fertilizer application soil collected Hawassa Zuria district soil.

Source of Variation	DF	Mean of Square						
		PH(cm)	LA	NB	NCPP	NFrPC	FD(mm)	MFY(g/plant)
Azolla	3	2518.52 <sup>**</sup>	280.38 <sup>**</sup>	12.14 <sup>ns</sup>	18.35 <sup>*</sup>	2.68 <sup>**</sup>	105.91 <sup>ns</sup>	522981.34 <sup>**</sup>
Nitrogen	3	1539.35 <sup>**</sup>	266.48 <sup>ns</sup>	96.47 <sup>**</sup>	53.35 <sup>**</sup>	2.82 <sup>ns</sup>	115.46 <sup>*</sup>	586017.04 <sup>**</sup>
Azolla * Nitrogen	9	114.33 <sup>**</sup>	5.79 <sup>**</sup>	3.54 <sup>**</sup>	4.35 <sup>ns</sup>	0.40 <sup>*</sup>	9.17 <sup>**</sup>	43536.19 <sup>**</sup>
Error	32	14.06	0.74	2.08	2.18	0.18	6.40	4253.62

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , PH: plant height, LA: leaf area, NCPP: number of cluster per plant, NFrPC: number of fruit per cluster, NB: number of branch, FD: fruit diameter and MFY: marketable fruit yield.

Appendix Table 3. 3: Mean squares of tomato growth parameters influenced by Azolla dry biomass and inorganic N fertilizer application in the soil collected from Wondo Genet district soil.

Source of Variation	DF	Mean of Square						
		PH	LA	NB	NCPP	NFPC	FD(mm)	MFY(g/plant)
Azolla	3	693.46 <sup>**</sup>	255.06 <sup>**</sup>	7.81 <sup>ns</sup>	14.91 <sup>*</sup>	4.66 <sup>**</sup>	63.67 <sup>**</sup>	59942.55 <sup>ns</sup>
Nitrogen	3	1266.46 <sup>**</sup>	166.68 <sup>**</sup>	38.25 <sup>*</sup>	53.07 <sup>**</sup>	4.45 <sup>ns</sup>	76.70 <sup>ns</sup>	192023.63 <sup>**</sup>
Azolla * Nitrogen	9	43.45 <sup>**</sup>	19.00 <sup>**</sup>	6.94 <sup>**</sup>	3.21 <sup>**</sup>	0.39 <sup>*</sup>	64.84 <sup>**</sup>	2101.63 <sup>**</sup>
Error	32	12.71	1.62	4.89	1.65	0.08	11.49	35754.43

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , PH: plant height, LA: leaf area, NCPP: number of cluster per plant, NFPC: number of fruit per cluster, NB: number of branch, FD: fruit diameter and MFY: marketable fruit yield.

Appendix Table 4. 1: Mean squares of tomato growth and yield parameters influenced by Azolla dry biomass and inorganic N fertilizer application in the Hawassa Zuria district in combined 2 years data(2022/23 and 2023/24).

Source of Variation	DF	Mean of Square								
		PH	NCPP	NFrPC	AFW(g)	NFPP	NLPP	NB	MFY(t/ha)	TFY(t/ha)
Replication	4	20.25 <sup>ns</sup>	6.94 <sup>ns</sup>	0.44 <sup>ns</sup>	0.67 <sup>ns</sup>	7.52 <sup>ns</sup>	15.44 <sup>ns</sup>	0.52 <sup>ns</sup>	0.46 <sup>ns</sup>	2.39 <sup>ns</sup>
Year	1	464.67 <sup>ns</sup>	1704.08 <sup>ns</sup>	19.87 <sup>ns</sup>	273.44 <sup>ns</sup>	716.88 <sup>ns</sup>	102.08 <sup>ns</sup>	6.02 <sup>ns</sup>	29.27 <sup>ns</sup>	129.76 <sup>ns</sup>
Azolla	3	764.89 <sup>**</sup>	72.96 <sup>**</sup>	23.14 <sup>**</sup>	726.69 <sup>**</sup>	527.28 <sup>**</sup>	273.18 <sup>**</sup>	3.69 <sup>**</sup>	190.98 <sup>**</sup>	945.51 <sup>**</sup>
N	3	186.22 <sup>**</sup>	33.14 <sup>**</sup>	16.53 <sup>**</sup>	769.79 <sup>**</sup>	159.84 <sup>**</sup>	273.18 <sup>**</sup>	4.24 <sup>**</sup>	256.51 <sup>**</sup>	1636.19 <sup>*</sup>
Year*Azolla	3	117.13 <sup>ns</sup>	60.46 <sup>ns</sup>	6.03 <sup>ns</sup>	775.76 <sup>ns</sup>	12.16 <sup>ns</sup>	3.68 <sup>ns</sup>	1.59 <sup>ns</sup>	1.73 <sup>ns</sup>	2.92 <sup>ns</sup>
Year*N	3	34.45 <sup>ns</sup>	21.72 <sup>ns</sup>	3.74 <sup>ns</sup>	109.74 <sup>ns</sup>	30.45 <sup>ns</sup>	2.24 <sup>ns</sup>	0.29 <sup>ns</sup>	1.539 <sup>ns</sup>	3.52 <sup>ns</sup>
Azolla * N	9	32.14 <sup>**</sup>	1.85 <sup>**</sup>	0.47 <sup>*</sup>	64.84 <sup>**</sup>	12.04 <sup>**</sup>	18.48 <sup>**</sup>	0.56 <sup>**</sup>	16.12 <sup>**</sup>	17.12 <sup>**</sup>
Year*Azolla*N	9	38.69 <sup>ns</sup>	11.49 <sup>ns</sup>	1.71 <sup>ns</sup>	38.26 <sup>ns</sup>	9.49 <sup>ns</sup>	1.98 <sup>ns</sup>	0.31 <sup>ns</sup>	0.73 <sup>ns</sup>	1.71 <sup>ns</sup>
Error	60	6.46	3.38	0.37	6.68	3.09	3.15	0.38	1.84	2.25

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , PH: plant height, NCPP: number of clusters per plant, NFrPC: number of fruits per cluster, AFW(g): average fruit weight per plant and NFPP: number of fruits per plant, NLPP: number of leaves per plant, NB= number of branches, MFY(t/ha)=marketable fruit per hectare and TFY(t/ha)= total fruit yield per hectare

Appendix Table 4. 2: Mean squares of tomato growth and yield parameters influenced by Azolla dry biomass and inorganic N fertilizer application in the Wondo Genet district in combined 2 years data(2022/23 and 2023/24).

Source of Variation	DF	Mean of Square								
		PH	N CPP	NFrPC	AFW(g)	NFPP	NLPP	NB	MFY(t/ha)	TFY(t/ha)
Replication	4	32.58 <sup>ns</sup>	1.13 <sup>ns</sup>	1.18 <sup>ns</sup>	4.27 <sup>ns</sup>	0.58 <sup>ns</sup>	0.23 <sup>ns</sup>	1.02 <sup>ns</sup>	2.58 <sup>ns</sup>	3.34 <sup>ns</sup>
Year	1	471.47 <sup>ns</sup>	1644.08 <sup>ns</sup>	21.56 <sup>ns</sup>	276.12 <sup>ns</sup>	712.48 <sup>ns</sup>	111.12 <sup>ns</sup>	8.03 <sup>ns</sup>	32.12 <sup>ns</sup>	136.81 <sup>ns</sup>
Azolla	3	1121.72 <sup>**</sup>	148.68 <sup>**</sup>	22.94 <sup>**</sup>	824.18 <sup>**</sup>	459.14 <sup>**</sup>	289.38 <sup>**</sup>	7.78 <sup>**</sup>	228.88 <sup>**</sup>	255.06 <sup>**</sup>
N	3	357.56 <sup>**</sup>	69.41 <sup>**</sup>	14.61 <sup>**</sup>	1267.23 <sup>**</sup>	291.64 <sup>**</sup>	282.43 <sup>**</sup>	6.42 <sup>**</sup>	414.12 <sup>**</sup>	464.04 <sup>**</sup>
Year*Azolla	3	119.52 <sup>ns</sup>	64.82 <sup>ns</sup>	6.03 <sup>ns</sup>	778.73 <sup>ns</sup>	14.11 <sup>ns</sup>	3.29 <sup>ns</sup>	2.19 <sup>ns</sup>	1.94 <sup>ns</sup>	4.14 <sup>ns</sup>
Year*N	3	36.17	23.61 <sup>ns</sup>	2.81 <sup>*</sup>	111.74 <sup>*</sup>	27.75 <sup>*</sup>	2.81 <sup>ns</sup>	0.47 <sup>ns</sup>	1.77 <sup>ns</sup>	4.28 <sup>ns</sup>
Azolla * N	9	1072.67 <sup>*</sup>	9.18 <sup>**</sup>	0.82 <sup>**</sup>	95.43 <sup>**</sup>	14.39 <sup>**</sup>	16.41 <sup>*</sup>	0.79 <sup>*</sup>	25.12 <sup>**</sup>	27.34 <sup>**</sup>
Year*Azolla*N	9	34.95 <sup>ns</sup>	12.77 <sup>ns</sup>	1.92 <sup>ns</sup>	41.29 <sup>ns</sup>	10.88 <sup>ns</sup>	2.14 <sup>ns</sup>	0.51 <sup>ns</sup>	0.99 <sup>ns</sup>	2.92 <sup>ns</sup>
Error	60	7.62	2.62	1.12	10.82	2.43	6.31	0.54	3.15	3.26

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , PH: plant height, NCPP: number of clusters per plant, NFrPC: number of fruits per cluster, AFW(g): average fruit weight per plant and NFPP: number of fruits per plant, NLPP: number of leaves per plant, NB= number of branches, MFY(t/ha)=marketable fruit per hectare and TFY(t/ha)= total fruit yield per hectare.

Appendix Table 5. 1: Mean Squares values of ANOVA for parameters measured  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and total nitrogen mineralization (TNM) evaluated for 56 days of incubation in the laboratory the soil collected from Hawassa Zuria districts.

Source of variations	Degree of freedom	$\text{NH}_4^+$	$\text{NO}_3^-$	Total NM
Azolla	3	435.54 <sup>**</sup>	545.85 <sup>**</sup>	1954.86 <sup>**</sup>
Incubation day	7	106.31 <sup>**</sup>	109.34 <sup>**</sup>	429.43 <sup>**</sup>
Azolla*Incubation day	21	27.14 <sup>**</sup>	24.40 <sup>**</sup>	102.02 <sup>**</sup>
Error	64	0.23	0.87	1.81

ns: not significant at  $P \leq 0.05$ , \*significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ ,

Appendix Table 5. 2: Mean Squares values of ANOVA for parameters measured  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and total nitrogen mineralization (TNM) evaluated for 56 days of incubation in the laboratory the soil collected from Wondo Genet districts.

Source of variations	Degree of freedom	$\text{NH}_4^+$	$\text{NO}_3^-$	Total NM
Azolla	3	346.70 <sup>**</sup>	318.13 <sup>**</sup>	1372.96 <sup>**</sup>
Incubation day	7	335.73 <sup>**</sup>	347.92 <sup>**</sup>	1364.58 <sup>**</sup>
Azolla*Incubation day	21	60.60 <sup>**</sup>	50.26 <sup>**</sup>	220.50 <sup>**</sup>
Error	64	0.32	0.52	1.23

ns: not significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.05$ , \* significant at  $P \leq 0.01$ , \*\*

