TABLE OF CONTENTS

1.0	IN	FRODUCTION	1
1	.1 Nee	d for improved fertilization methods	1
1	.2 Nit	rogen forms taken up by crops	1
1	.3 Lii	nitations of nitrate based fertilizers	2
1	.4 Ag	conomic requirements and economic importance of barley	2
1	.5 Prii	nciple of CULTAN fertilization technique	3
1	.6 Dil	emma of inadequate and excess nitrogen nutrition	3
1	.7 Pot	ential of Nitrification inhibitor incorporation in CULTAN	4
1	.8 Me	rits of CULTAN fertilization technique	4
1	.9 Obj	ectives of the study	5
2.0	MA	ATERIALS AND METHODS	7
2.1	Det	ermination of soil-injected liquid NH4 ⁺ stability	7
2.1.1	Exp	perimental site location and crop growth conditions	7
2.1.2	Exp	perimental design	7
2.1.3	Nit	rogen treatments and sampling intervals	8
2.1.4	Gro	with nutrient application and nitrification inhibitor incorporation	9
2.1.5	Cro	p sample categories at different intervals	11
2.1.6	Gro	owth parameters, grain yield and yield components	11
2.1.7	Cro	p analyses	12
	2.1.7	1 Chlorophyll analysis	12
	2.1.7	2 Water-soluble carbohydrate (WSC) analysis	12
	2.1.7	.3 Total carbon and nitrogen concentrations	13
	2.1.7	.4 Nitrate analysis	13
	2.1.7	.5 Cation and anion analysis	13
	2.1.7	.6 Organic acid analysis	13
2.1.8 Soil and		l analyses	14
	2.1.8	1 Ammonium and nitrate determination	14
	2.1.8	2 Potassium and phosphorus determination	14
	2.1.8	.3 Cation exchange capacity	14

2	2.1.8.4 Soil pH	15
2.1.9	Data analysis	15
2.2 (Characterization of bacterial community responses to	
C	CULTAN fertilization	16
2.2.1	Soil samples analyzed for bacterial occurrence	16
2.2.2	Bacterial DNA extraction from environmental soils	16
2.2.3	DNA amplification, purification and quantification	17
2.2.4	Single-strand conformation polymorphism (SSCP) technique	18
2	2.2.4.1 Reagents and equipments used in SSCP technique	18
2	2.2.4.2 Generation of the single-stranded DNA	18
2	2.2.4.3 SSCP gel silver staining and development	19
2	2.2.4.4 Band selection, excision and soaking	19
2.2.5	DNA cloning (ligation and transformation)	20
2.2.6	DNA Sequencing	21
2	2.2.6.1 Sequencing reagents and equipment	21
2	2.2.6.2 Sequencing procedure	21
2.2.7	Statistical Analyses	22
2	2.2.7.1 Digital image analysis	22
2	2.2.7.2 Sequence analyses	22
23	Fyaluation of the biodiversity of ammonia oxidizing bacteria	24
2.0	Evaluation of the biodiversity of animonia oxidizing bacteria	2-1
2.3.1	Detection of ammonia oxidizing bacteria (AOB)	24
2.3.2	Selection and optimization of amoA primers	24
2.3.3	Selection of AOB for use as positive control	24
2.3.4	PCR amplification of AOB with amoA primers	24
2.3.5	Development of amoA SSCP gels	25
2.3.6	Band selection, ligation, transformation and sequencing	25
2.3.7	Statistical analysis	25

2.4	Ammonia oxidizing bacteria population abundance in		
	CULTAN-fertilized soils	26	
2.4.1	Quantification of N. multiformis gene copies	26	
2.4.2	Real-Time PCR optimization	26	
2.4.3	Generation of <i>amoA</i> standard curves	27	
2.4.4	Threshold cycle determination	27	
2.4.5	Melting point curves of amoA genes	27	
2.4.5	Statistical analysis	27	
2.5	Effect of temperature on CULTAN-fertilized barley	28	
2.5.1	Experimental site and crop growth conditions	28	
2.5.2	Experimental design	28	
2.5.3	Temperature and nitrogen treatments	28	
2.5.4	Soil sampling zones	29	
2.5.5	Carbon Exchange Rates (CERs) and SPAD measurements	31	
2.5.6	Shoot and root biomass estimates	31	
2.5.7	Crop analyses	32	
2.5.8	Soil analyses	32	
2.5.9	Data analysis	33	
3.0	RESULTS	34	
3.1	Stability of soil-injected liquid NH_4^+	34	
3.1.1	Soil physical and chemical characteristics	34	
3.1.2	Concentration of soil-injected NH ₄ ⁺	36	
3.1.3	Concentration of NO_3^- in the soil	37	
3.1.4	Potential of nitrogen loss in CULTAN-fertilized soils	38	
3.1.5	Influence of CULTAN fertilization on soil pH	40	

iii

3.1.6	Phosphorus and potassium availability under CULTAN	42
3.1.7	Crop growth and yield responses to CULTAN-fertilization	44
	3.1.7.1 Biomass accumulation and relative growth rates (RGRs)	46
	3.1.7.2 Barley grain yield and yield-forming factors	48
3.1.8	Chemical composition of crops fertilized with different N forms	50
3.1.8	.1 Crop nitrogen uptake and partitioning	50
3.1.8	.2 Shoot mineral concentrations	52
3.1.8	.3 Leaf chlorophyll and carotenoid concentrations	55
3.1.8	.4 Sugar concentrations	57
3.1.8	.5 Organic acid concentrations	58
3.2	Bacterial community diversity responses to CULTAN fertilization	60
3.2.1	Bacterial community detection	60
3.2.1 3.2.2	Bacterial community detection Spatial dynamics of detected bacterial communities	60 60
3.2.13.2.23.2.3	Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities	60 60 64
3.2.13.2.23.2.33.2.4	Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities	60 60 64 66
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 	Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities	60 60 64 66 66
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 	Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups	60 64 66 66 67
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 3.3 	 Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups Biodiversity of AOB associated with CULTAN-fertilized soils	60 64 66 66 67 69
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 3.3 3.3.1 	 Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups Biodiversity of AOB associated with CULTAN-fertilized soils Spatial changes of ammonia oxidizing bacteria under 	60 64 66 66 67 69
3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 3.3 3.3.1	 Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups Biodiversity of AOB associated with CULTAN-fertilized soils Spatial changes of ammonia oxidizing bacteria under CULTAN fertilization 	60 64 66 67 69
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 3.3 3.3.1 3.3.2 	 Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups Biodiversity of AOB associated with CULTAN-fertilized soils Spatial changes of ammonia oxidizing bacteria under CULTAN fertilization Temporal dynamics of ammonia oxidizing bacteria under 	 60 64 66 67 69
 3.2.1 3.2.2 3.2.3 3.2.4 3.2.5 3.2.6 3.3 3.3.1 3.3.2 	 Bacterial community detection Spatial dynamics of detected bacterial communities Temporal dynamics of detected bacterial communities Characterization of CULTAN-associated bacterial communities Composition of CULTAN-associated bacterial communities Phylogenetic relationships of characterized CULTAN bacterial groups Biodiversity of AOB associated with CULTAN-fertilized soils Spatial changes of ammonia oxidizing bacteria under CULTAN fertilization Temporal dynamics of ammonia oxidizing bacteria under CULTAN fertilization 	 60 64 66 67 69 71

3.4	Estimate of AOB population abundance in	
	CULTAN-fertilized soils	75
3.5	Growth temperature effect on grain yield of	
	CULTAN-fertilized barley	79
3.5.1	Barley growth duration and shoot height	79
3.5.2	Biomass accumulation and partitioning	80
3.5.3	Grain yield and yield forming-factors	81
3.5.4	Response of yield-forming factors to temperature	
	and CULTAN-fertilization	83
3.5.5	Carbon exchange rates (CERs) and SPAD	83
3.5.6	Soil and crop nutrient concentration	85
4	DISCUSION	89
4.1	Stability of soil injected liquid NH_4^+	89
4.1.1	Establishment of NH_4^+ sorption-complex zones	89
4.1.2	Nitrogen forms available in CULTAN-fertilized soils	92
4.1.3	Barley root growth responses to CULTAN fertilization	94
4.1.4	Barley aerial growth responses to CULTAN fertilization	96
4.1.5	Possible mechanisms involved in mixed N nutrition under CULTAN	99
4.2	Bacterial community responses to CULTAN-fertilization	102
4.2.1	Occurrence of bacterial communities within CULTAN-fertilized soils	102
4.2.2	Functional responses of AOB in CULTAN-fertilized soils	103
4.2.3	Effect of bacterial communities on N form in CULTAN fertilized soils	104
4.2.4	Bacterial community shift occurrence in CULTAN fertilized soils	105
4.2.5	Possible influences of soil pH on microbial community	107

v

4.2.6	Bacterial community structure restoration from CULTAN-effects	108
4.2.7	Potential use of nitrification inhibitors to suppress AOB activity in	
	CULTAN fertilization	109
4.3	Ammonia oxidizing bacteria population abundance in	
	CULTAN-fertilized soils	111
4.3.1	Relative AOB population abundance in CULTAN fertilized soils	111
4.3.2	Potential of ammonia oxidation by non-Proteobacteria	112
4.4	Effect of growth temperatures on CULTAN-fertilized	
	Barley crop grain yield formation	114
4.4.1	Effects of temperature on CULTAN-fertilized barley growth responses	114
4.4.2	Temperature effects on grain yield of CULTAN-fertilized barley	114
4.4.3	Fate of CULTAN-fertilizer upon injection into soil	115
4.4.4	Potential of CULTAN-fertilization for crop production	116
4.4.5	Possible effects of temperature on CULTAN-fertilization	117
5.	SUMMARY	119
6.	ZUSAMMENFASSUNG	121
7.	REFERENCES	124
8.	ACKNOWLEDGEMENT	143
9.	DEDICATION	145
10.	CURRICULUM VITAE	146

vi