



Reserach Article

Spatial and Temporal Changes of Soil Nitrogen Contents and Determination of Nitrogen Resources by Natural Isotope Technique in Agricultural Lands

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Abstract

In the current study soil samples were taken at three different periods at 51 points in Çanakkale-Kumkale Plain (Troy). Soil total nitrogen (N), nitrate and ammonium contents were conducted to determine the spatial and temporal distribution of soil nitrogen. Additionally, possible sources of soil nitrogen were evaluated using $\delta^{15}\text{N}$ isotope tools. According to $\delta^{15}\text{N}$ results, the main source of soil nitrogen was inorganic nitrogen fertilizers in cotton, wheat, and tomato-grown soils in April. In December, most of the soil $\delta^{15}\text{N}$ was of both organic and mineral origin. The value of soil $\delta^{15}\text{N}$ values increased in July because of enhanced organic matter mineralization in the area. The $\delta^{15}\text{N}$ values of the great majority of soils were between 5‰ and 10 ‰ which indicates that N was derived from organic materials. The reason for the low $\delta^{15}\text{N}$ values in April was due to the use of excess mineral fertilizers for cotton, maize, tomato, pepper, sunflower, and wheat crops.

Keywords: $\delta^{15}\text{N}$, nitrogen, carbon, land use, isotope, soil

Tarım Arazilerinde Toprak Azot İçeriklerinin Mekansal ve Zamansal Değişimleri ve Azot Kaynaklarının Doğal İzotop Tekniği ile Belirlenmesi.

Öz

Bu çalışmada Çanakkale-Kumkale Ovası'nda (Truva) 51 farklı noktadan, üç farklı dönemde toprak örnekleri alınmıştır. Toprakta bulunan azotun, mekansal ve zamansal dağılımını belirlemek için topraklarda toplam azot (N), nitrat-N ve amonyum-N içerikleri belirlenmiştir. Ayrıca topraktaki azotun kaynaklarını belirleyebilmek için, toprakların doğal $\delta^{15}\text{N}$ izotop değerleri belirlenmiştir. $\delta^{15}\text{N}$ sonuçlarına göre Nisan ayında pamuk, buğday ve domates yetiştirilen topraklarda toprak azotunun ana kaynağı inorganik azotlu gübreler olmuştur. Aralık ayında ise çoğu toprakta azotun kaynağı organik ve mineral kökenlidir. Artan organik madde mineralizasyonu nedeniyle, örnekleme alanlarındaki toprak $\delta^{15}\text{N}$ değerleri Temmuz ayında artış göstermiştir. Toprakların büyük çoğunluğunun $\delta^{15}\text{N}$ değerleri 5‰ ile 10‰ arasında olup, bu da topraktaki N'nin organik orijinli olduğunu göstermektedir. Nisan ayında $\delta^{15}\text{N}$ değerlerinin düşük olmasının nedeni, pamuk, mısır, domates, biber, ayçiçeği ve buğday bitkileri için fazla mineral gübre kullanılmasıdır.

Anahtar Kelimeler: : $\delta^{15}\text{N}$, azot, karbon, arazi kullanımı, izotop, toprak

Introduction

Nitrogen (N) is an essential element for all organisms, and it can limit the net primary productivity of aquatic and terrestrial ecosystems (Cui et al., 2013) and one of the most required nutrients for plant growth. Excessive and uncontrolled use of the nitrogen element as fertilizer leads to very serious environmental problems (Brevik et al., 2015; Vejan et. al., 2016). Since the 1960s, the use of nitrogenous fertilizers in agricultural land has started to increase and approximately 77 million tons of nitrogenous fertilizer were used worldwide in 1990, and up to 178 million tons of ammonia was produced in 2021 (Mordor Intelligence, 2021). In Turkey, the use of fertilizers was 6.3 million tons

and 31% of this was urea, 13% was diammonium sulfate, and 11% was ammonium sulfate (Anonymous, 2018). Besides chemical fertilizers, one of the major sources of nitrogen in the soil is the mineralization of organic matter and plant residues (Brady, 1996; Ünlü et al., 1999, Abbasi et al., 2015). Urban wastes (such as sewage) can also be sources of nitrogen for the soil (Alvarenga et al., 2016).

The natural abundance of ^{15}N of plants and soils has been used for gaining information into changes in nitrogen cycling (Högberg 1997; Robinson 2001; Vitousek 2004). Nitrogen isotopes provide information about the origin of nitrogen in the environment (forms of N sources) and the processes in which the N has participated (e.g., nitrification and denitrification) (Hyodo et al., 2013). $\delta^{15}\text{N}$ is used as an important indicator in determining the nitrogen dynamics in the ecosystem. The values of $\delta^{15}\text{N}$ vary according to the source of N. The $\delta^{15}\text{N}$ values of chemical fertilizers range from 0 to 5 ‰ and if $\delta^{15}\text{N}$ values are greater than 10 ‰ manure can be the primary N source. The nitrate originating from sewage is around $\delta^{15}\text{N} + 20\text{‰}$ and is very different from chemical fertilizers (Clark and Frits, 1997).

The aim of this study was to determine soil nitrogen changes depending on the season and cropping patterns and to identify the possible sources of soil nitrogen by using the natural isotope technique in the Troy area. For this purpose, soil samples were taken during three different periods (December 2002, April 2003, and July-2003) at 51 points in Çanakkale-Kumkale Plain (Troy). Soil N and $\delta^{15}\text{N}$ analyses were conducted to determine the spatial and temporal distribution of soil nitrogen and $\delta^{15}\text{N}$.

Materials And Methods

Study area

The study area is the Kumkale plain within the Troy National Park boundaries and covers an area of approximately 5260 ha (Figure 1). The national park is one of the most important and ancient archaeological sites in the northwest of Türkiye. The study area consists of flat, very lightly steep slopes with a slope of 0-3%. The soil texture classes in the surface layer of the study area varies from sandy to clay. According to Soil Survey Staff (1999), these soils were classified as Xerofluvents and Haploxererts; According to WRB (2006), it is classified as Fluvisol and Vertisol. Major crop types used in the rotation are wheat, corn, cotton, and tomatoes (Figure 2) in the region. The annual average precipitation of the research site was 600 mm and the annual mean temperature was 15.2 °C and it was located within the extensions of the Mediterranean climate zone.

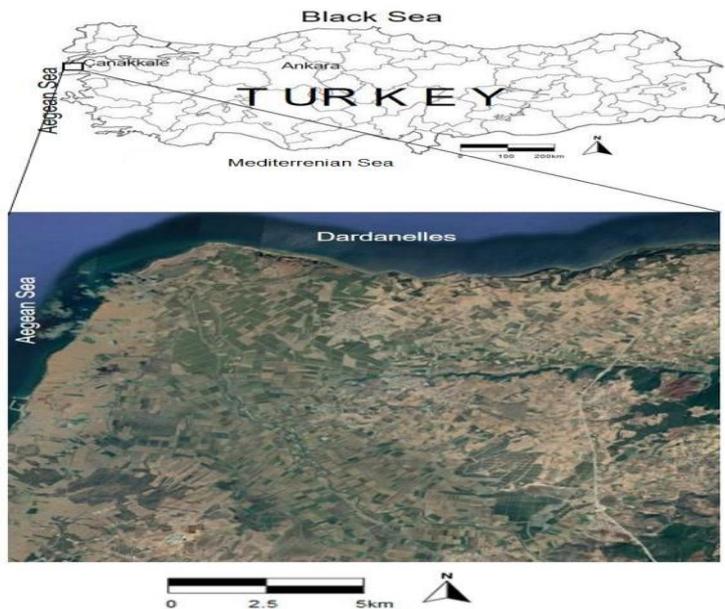


Figure 1. Map of the study area

Şekil 1. Çalışma alanının lokasyon haritası

Soil sampling and analysis

Soil samples were taken from 0-25 cm depths at three different times considering fertilizing and harvesting periods (December 2002, April 2003, and July 2003) from the same sampling points (Figure.3). The sampling points were determined by evaluating the aerial photographs, contours in the topographical map, and the large soil group map. The Global Position System (GPS) was used to record the location (in longitude and latitude) of the sampling points.

During the study, ArcView3.2 Geographic Information System software (GIS) was used to create terrestrial maps. Additionally, a 1/25000 scale topographical map, 1/30000 scale aerial photographs belonging to 1967, and a Soil Map of 1/100000 scale, have been used.

A total of 153 soil samples from 51 sampling points were taken and analyzed (Figure 3). The soil samples were air-dried at room temperature and sieved through a 2 mm sieve. Soil textures were determined by the Hydrometer method (Gee and Bauder, 1986). Soil samples were mixed with distilled water (1:2.5 soil: water ratio) and pH (Soil Survey Staff, 1996) and electrical conductivity (EC) values were determined (Soil Survey Staff, 1996). Cation exchange capacity (CEC) was determined by using sodium acetate (Chapman, 1965). Soil CaCO₃ content was measured by the Scheibler method (Schlichting and Blume, 1966).

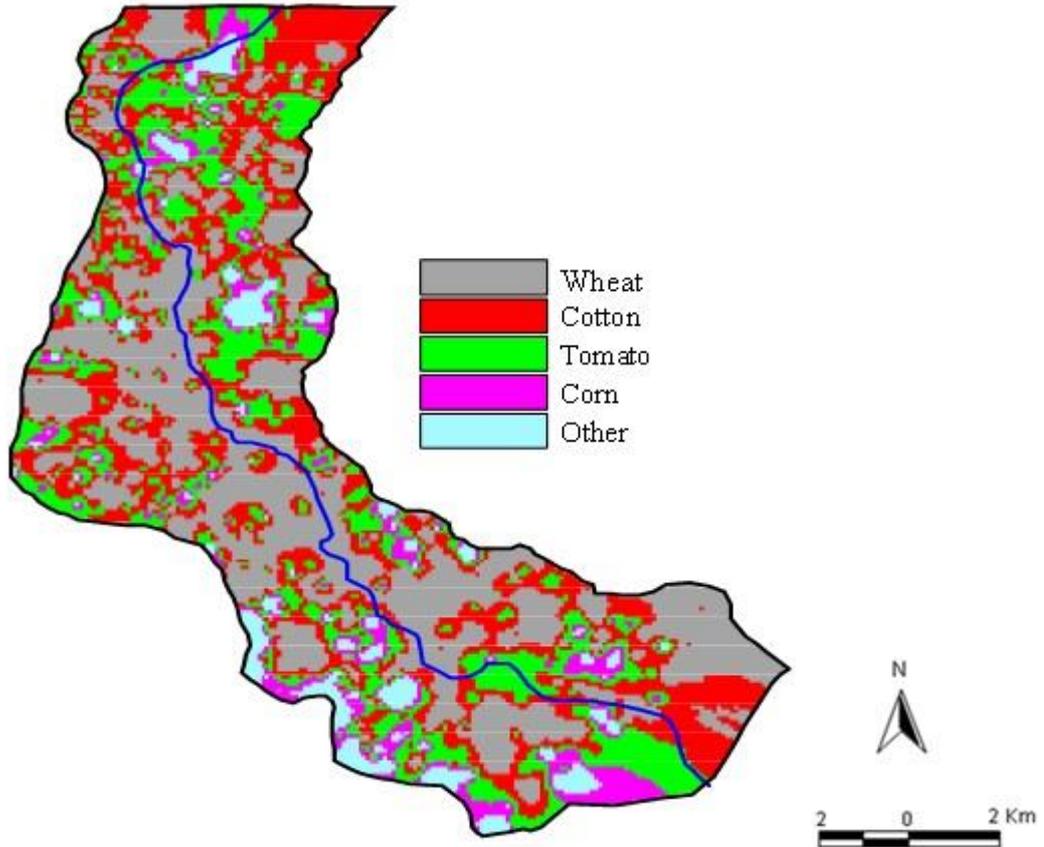


Figure 2. Cropping map in the study area (July)
Şekil 2. Çalışma alanının ekim haritası (Temmuz)

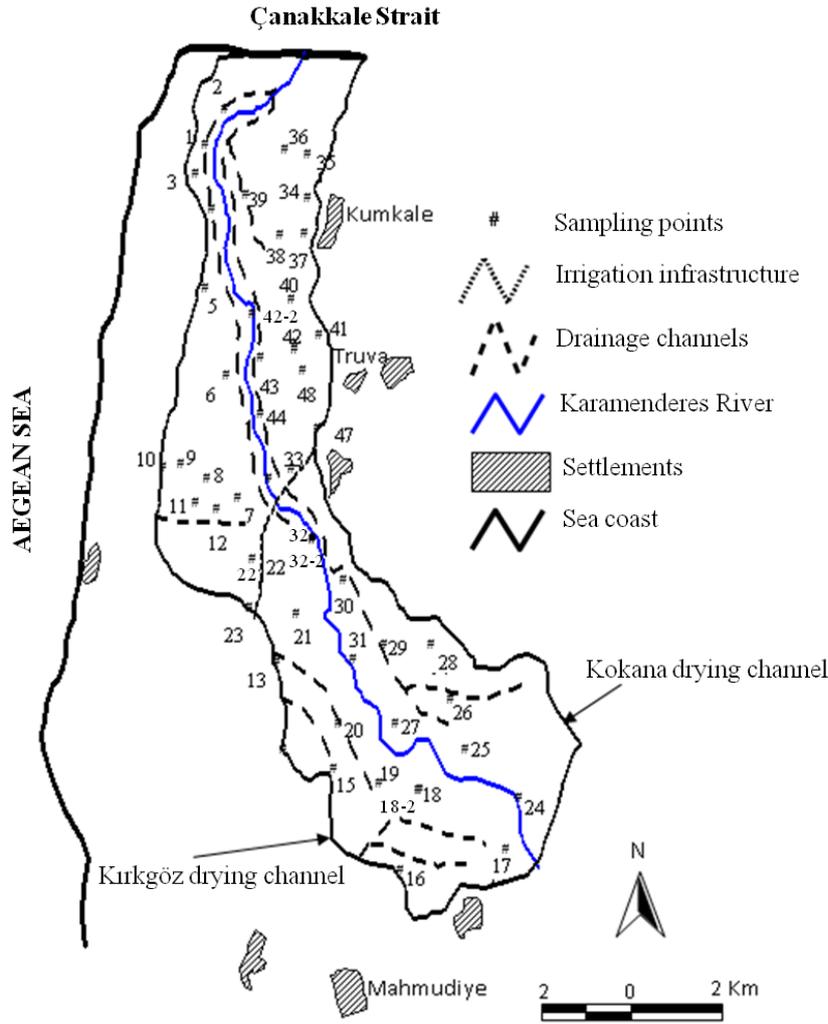


Figure 3. Soil sampling points at the study site
Şekil 3. Çalışma alanında toprak örnekleme noktaları

Total N, C and ¹⁵N analysis

The analyzes were carried out in the isotope laboratories of the Crop and Soil Science Department of Michigan State University, USA. The soil samples were air-dried and then sieved from 2 mm sieves. After finely grinding soils were weighed into the tin capsules nearest 0.0001 g. The total SOC and N values of the samples were determined according to the dry combustion method using the Carlo Erba C / N / H NA 1500 analyzer (Kirsten, 1983). Soil ¹⁵N analysis was conducted by using the EuropaScientific mass spectrometry model 2020 (IRMS). Calculations are made according to Yoneyama (1996).

¹⁵N contents of soils were calculated according to equation (1)

$$\delta^{15}\text{N} = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1000 \quad \text{equation (1)}$$

R_{sample} is the ¹⁵N/¹⁴N ratio of a soil sample and R_{standard} is the ¹⁵N/¹⁴N ratio of the standard atmospheric N₂

$$^{15}\text{N}\% (\text{atmosphere}) = 0.366303$$

Descriptive statistical analyzes of samples were done using SPSS (1988) program.

Results and Discussions

Descriptive statistics of some physico-chemical properties in soil samples are presented in Table 1. Soil pH values ranged from 7.1 to 8.01 with a mean of 7.52 which were classified as neutral and slightly alkaline. EC values were in the range of 30-320 µScm⁻¹, with a mean of 67 µScm⁻¹ and it was determined that these values did not indicate any salinity problems for agricultural production.

Table 1. Descriptive statistics for soil samples taken in December 2002.

Çizelge 1. Aralık 2002'de alınan toprak örnekleri için tanımlayıcı istatistikler.

Soil properties	Minimum	Maximum	Mean	C.V. (%)
pH	7.10	8.01	7.52	2.9
EC (µS/cm)	30	320	67	60
Soil organic carbon (%)	0.05	2.28	0.78	60
CaCO ₃ (%)	0.17	12.68	3.50	81
Clay (%)	8.13	54.29	24.87	41
Silt (%)	6.8	51.7	34.30	29
Sand (%)	7.8	78.13	40.80	39
CEC (cmol/kg)	3.04	30.12	11.54	38

The area is alluvial land and such soils often show large variations in their properties over short distances (Dengiz, 2020). There were high variations in soil properties (EC, SOC, CaCO₃, clay, silt, and sand) (Table 1).

Soil Nitrogen

The total N (TN) of soils was less than 0.3% in the majority of the area. The lowest TN value was 0.053% which was sampled in December (sample number 9) while the highest value was 0.33% which was sampled in July (sample number 19) in the pasture where the total organic carbon (TOC) value of this land reached 3.56% (Figures 4 and 5).

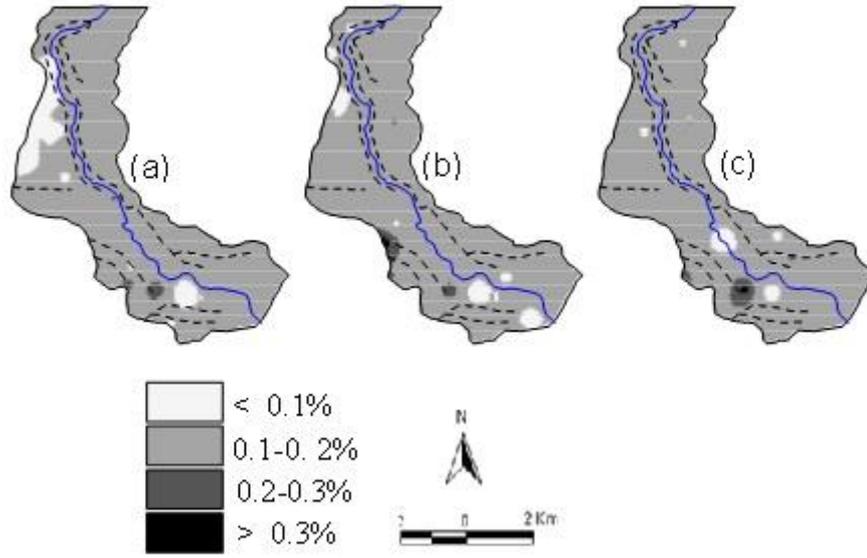


Figure 4. Changes of soil N concentrations in the study area (a: December-2002; b: April-2003; c: July-2003)

Şekil 4. Çalışma alanındaki toprak N konsantrasyonlarındaki değişimler (a: Aralık-2002; b: Nisan-2003; c: Temmuz-2003)

Since July is a drier month than April and December, it was thought that the amount of leaching from the soil is relatively less. The highest amount of $\text{NH}_4\text{-N}$ has been observed in the vegetable field ($640 \text{ kg NH}_4\text{-N ha}^{-1}$) where the nitrate content of this soil was $50 \text{ kg NO}_3\text{-N ha}^{-1}$ (Table 2) and SOC concentration was 3.56%. Sunflower planted and fertilized agricultural soils (sample 26) had also high (560 kg N ha^{-1}) inorganic nitrogen content which consist of $330 \text{ kg ha}^{-1} \text{ NH}_4$ and $230 \text{ kg ha}^{-1} \text{ NO}_3$. The high amount of nitrate content in the soil (number 37) sample taken after the tomato harvest was an indication of excessive commercial fertilization in these areas.

According to the results of total nitrogen and inorganic nitrogen analysis in July soil samples, a significant part of the inorganic nitrogen was ammonium. However, at the sampling points 41, 44, 45, 48, and 42-2, all of the inorganic nitrogen was nitrate (Table 2). Soil textures in these locations were loam and sandy loam and due to coarse texture and adequate aeration, the nitrate-N form was dominant. Since July is the dry season in this area, nitrate leaching did not occur, and nitrate remained in the soil. The content of inorganic nitrogen in soils varies according to the differences in soil characteristics (especially texture), land use types, and management techniques. The presence of areas where inorganic nitrogen exceeds 5% indicates excessive nitrogen fertilization in these areas.

Table 2. Soil inorganic nitrogen contents, $\delta^{15}\text{N}$ values, and crop types
Çizelge 2. Toprak inorganik azot içerikleri, $\delta^{15}\text{N}$ değerleri ve bitki türleri

SN*	ST	NH ₄		NO ₃	TIN*	δ ¹⁵ N			Crops		
		kg ha ⁻¹				‰					
		July	Dec.	April	July	December	April	July			
1	CL	190	40	230	8.2	5.3	5.4	Wheat	Wheat	Wheat	
2	CL	100	150	250	16.9	1.6	6.2	Cotton		Tomato	
3	L	90	200	290	6.1	-0.3	4.1	Tomato		Tomato	
4	L	90	160	260	5.5	0.3	4.9	Cotton		Cotton	
5	SL	110	120	230	5.7	12.4	5.8	Cotton		Cotton	
6	CL	110	30	140	5.6	2.5	5.0	Wheat	Wheat	Wheat	
7	SCL	80	50	130	7.2	17.2	6.2	Wheat		Cotton	
8	L	110	30	140	7.5	1.5	5.9	Wheat	Wheat	Wheat	
9	L	100	120	220	2.1	8.4	6.8	Wheat		Cotton	
10	C	90	90	190	7.8	3.6	5.9	Sunflower		Corn	
11	C	120	30	150	8.0	4.7	8.7	Wheat	Wheat	Wheat	
12	CL	160	60	220	7.1	3.9	5.9	Sunflower	Crops	Wheat	
13	CL	100	50	150	8.2	8.9	5.8	Cotton		Cotton	
14	C	60	80	140	6.8	5.3	9.2	Sunflower		Corn	
15	L	170	60	230	6.9	7.9	6.6	Vegetable		Corn	
16	SiC										
16	L	140	160	300	8.8	6.8	6.7	Corn		Vegetable	
17	SL	90	20	110	5.9	4.0	5.7	Vegetable		Cotton	
18-1	SL	60	30	90	5.2	6.6	9.0	Poplar	Poplar	Poplar	
18-2		90	70	160	5.4	7.7	9.1	Cotton		Cotton	
19	CL	640	50	690	7.7	4.3	5.8	Vegetable	Vegetable	Vegetable	
20	L	120	150	260	6.9	5.4	7.4	Cotton		Cotton	
21	L	100	60	160	7.6	4.8	6.4	Cotton	Wheat	Wheat	
22	SCL	110	40	160	7.6	6.8	7.2	Cotton		Tomato	
23	SiC	250	60	320	6.2	5.7	6.1	Cotton	Wheat	Wheat	
24	SL	90	10	110	9.6	6.3	6.0	Wheat	Wheat	Wheat	
25	SL	60	100	160	7.4	7.3	6.7	Vegetable		Cotton	
26	L	330	230	560	7.2	4.7	7.9	Wheat	Sunflower	Sunflower	
27	SL	80	30	110	6.4	9.7	6.6	Vegetable	Wheat	Wheat	
28	CL	90	20	110	6.6	5.6	6.2	Cotton	Wheat	Wheat	
29	CL	70	60	130	6.8	6.3	6.5	Vegetable		Sunflower	
30	L	110	20	130	6.9	7.6	6.5	Wheat	Wheat	Wheat	
31	L	110	30	150	7.4	9.2	5.3	Cotton		Cotton	
32-1	L	110	80	200	5.5	4.8	5.0	Vegetable		Cotton	
32-2	L	100	30	130	10.3	3.4	4.9	Wheat	Wheat	Wheat	
33	SL	90	190	280	6.3	6.3	7.3	Cotton		Cotton	
34	CL	160	40	200	6.8	6.4	6.5	Vegetable	Vegetable	Tomato	
35	CL	90	20	110	6.4	8.0	9.5	Wheat	Wheat	Wheat	
36	L	80	100	180	6.3	6.9	6.8	Corn		Cotton	
37	CL	60	330	400	6.3	6.5	6.8	Tomato		Cotton	
38	L	10	120	130	5.4	8.8	6.7	Cotton		Tomato	
39	CL	120	30	150	7.5	7.3	7.8	Wheat	Wheat	Wheat	
40	L	30	10	30	8.3	7.8	13.4	Wheat	Wheat	Wheat	
41	L	0	10	10	6.6	7.1	6.7	Wheat	Wheat	Wheat	
42-1	CL	160	10	170	-	8.9	8.8	Pasture	Pasture	Pasture	
42-2	SiC										
42-2	L	0	260	260	9.2	8.7	9.4				
43	L	90	60	150	11.0	13.6	11.6	Cotton		Cotton	
44	SL	0	70	70	18.0	6.9	7.8	Cotton		Sunflower	
44	SiC										
44	L	0	40	40	4.6	7.6	8.5	Cotton		Sunflower	
46	L	60	180	230	6.1	7.8	8.0	Tomato		Corn	
47	L	30	30	50	5.5	8.2	13.5	Wheat		Cotton	
48	L	0	90	90	6.7	6.9	7.2	Wheat		Cotton	

• SN: Sample number; ST: Soil texture; TIN: Total Inorganic Nitrogen

Soil organic carbon (SOC)

The majority of soil SOC values were in the range of 1-2% while in some sampling points it exceeded >2 (Figure 4). Soil SOC was greater than %3 in numbers 19 (July sampling) and 24 (December, April, and July samplings). Similar to TN values, SOC values were also high in pasture soils where average SOC values in April and in July, were twice more than those of agricultural areas. The destruction of macroaggregates by tillage leads to an increase in the organic carbon required for microorganisms and as a result of increased microbial activity the SOC decreases in agricultural areas (Jastrow, 1996; Six et al., 1998). In general, there is an important relationship between total carbon and total nitrogen in the soil, and C:N ratio is the most important parameter used in defining this relationship. C:N ratios were between 9 and 19 in April and in July and between 9 and 23 in December (Figure 5). The incorporation of residues of high C:N ratio (>30) would favor immobilization at the first stage of decomposition. When C:N ratio is smaller than 20, the mineral nitrogen usually goes to the free state in the first stage of the decomposition process (Güzel, 1982). The C:N ratio of soils appears to be very low in July (Figure 6c) and higher in December (Figures 6a and b). One of the reasons for the high C:N ratio of soil in December is the addition of plant residues to the soil after harvest. The reason for the low C:N ratio in July was the application of inorganic N fertilizers and organic matter mineralization (samples of 3, 6, 7, 9, 10, 12, 14, 16, 20).

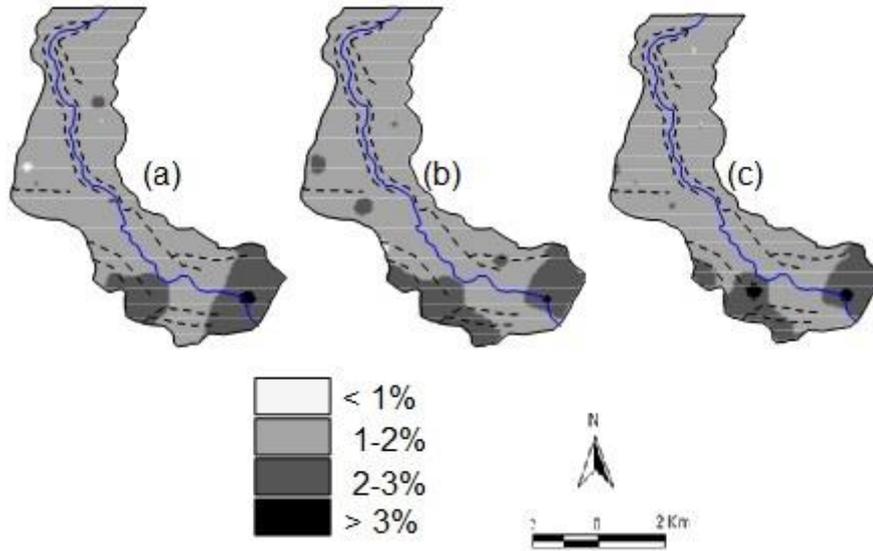


Figure 5. Soil organic carbon concentration changes during the year (a: December 2002; b: April 2003; c: July 2003)

Şekil 5. Toprak organik karbon konsantrasyonu değişimleri (a: Aralık 2002; b: Nisan 2003; c: Temmuz 2003)

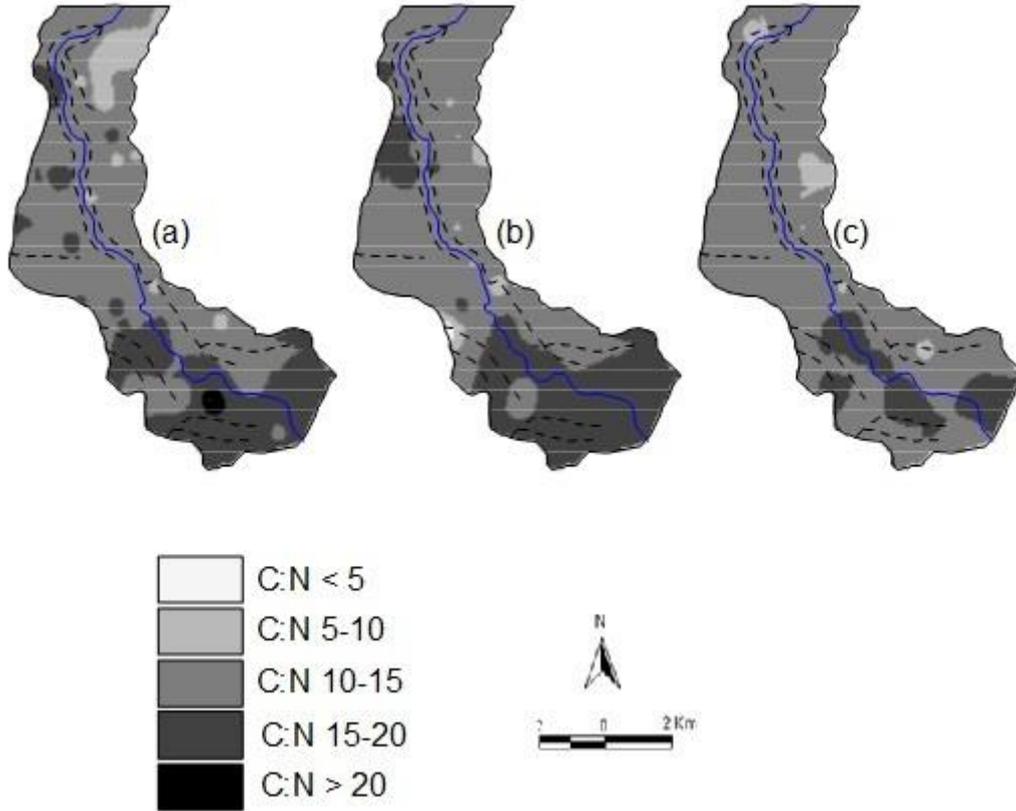


Figure 6. Soil C:N changes during the year (a: December 2002; b: April 2003; c: July 2003)
Şekil 6. Toprak C:N değişimleri (a: Aralık 2002; b: Nisan 2003; c: Temmuz 2003)

Soil $\delta^{15}\text{N}$

The results of soil $\delta^{15}\text{N}$ analysis in the study area are presented in Table 2. A total of 14 soil samples had $\delta^{15}\text{N}$ values below 5‰ in April (Table 2) indicating that the source of nitrogen in these soils was inorganic N fertilization. The amount of soil nitrogen derived from inorganic N fertilizers was mainly present in cotton, wheat, and tomato-grown soils in April.

In December, it was determined that most of the soil $\delta^{15}\text{N}$ had both organic and mineral origins while few of them derived from inorganic N fertilizers such as samples 9 (tilled wheat stubble) and 45 (cotton stubble). The origin of organic N is the mineralization of harvest residues in the field. Excessive commercial fertilizer found in cotton stubble fields indicates that too much nitrogen fertilizer has been applied to cotton plants. The value of soil $\delta^{15}\text{N}$ values increased in July because of enhanced organic matter mineralization and only three locations (3, 4, and 322) had a value of $\delta^{15}\text{N} < 5\text{‰}$. In these areas, excessive soil inorganic N was found after the cultivation of wheat, tomatoes, and cotton plants. The $\delta^{15}\text{N}$ values of chemical fertilizers range from 0 to 5 ‰ (Figure 7). According to the literature, as the N resulting from chemical fertilizers increases, the value of $\delta^{15}\text{N}$ approaches zero (Shearer and Kohl, 1989; Van Kessel et al., 1994 Högberg, 1997). Vitoria et al. (2005) reported that total nitrogen isotopic compositions ($\delta^{15}\text{N}$ total) were very similar for all types of N fertilizers, ranging from -1.7 to +3.9‰ with a mean of +0.0‰. During the production of chemical nitrogenous fertilizers, atmosphere N_2 is converted to NH_3 form by combining with hydrogen at high temperatures then NH_3 is oxidized and converted into NO_3 . For this reason, $\delta^{15}\text{N}$ values of chemical fertilizers are similar to atmosphere $\delta^{15}\text{N}$ values (Clark and Fritz, 1997).

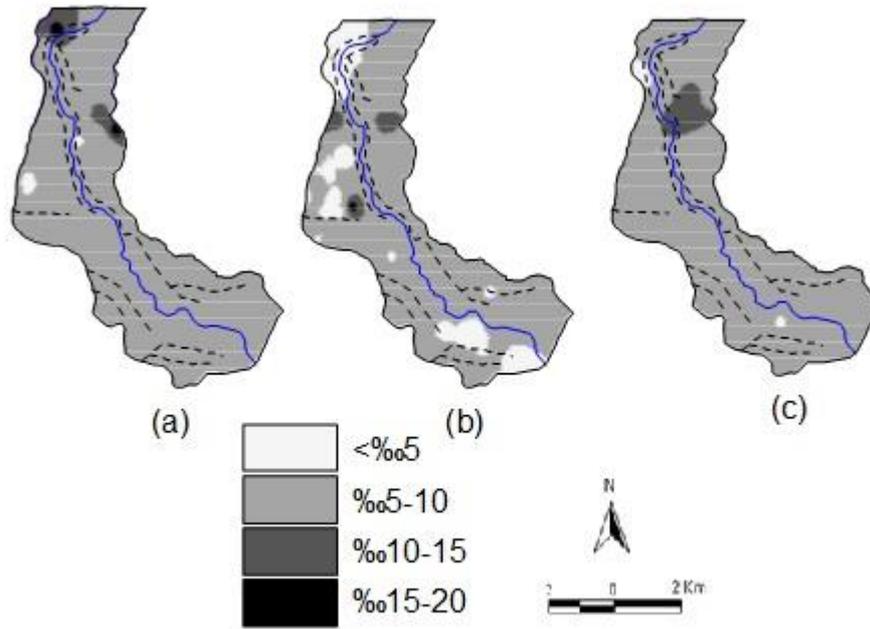


Figure 7. Soil $\delta^{15}N$ content changes during the year (a: December 2002; b: April 2003; c: July 2003)
Şekil 7. Toprak $\delta^{15}N$ içeriklerinin değişimi (a: Aralık 2002; b: Nisan 2003; c: Temmuz 2003)

The $\delta^{15}N$ values of the great majority of soils were between 5 and 10‰ (Table 2, Figure 7). In this case, the nitrogen was derived from organic materials such as plant residues, microbial biomass nitrogen, and organic fertilizers (Figure 7). If the total nitrogen content of the soil is low but the value of $\delta^{15}N$ is high ($>10\text{‰}$) (Figure 4), the nitrogen source may be animal manure (Figure 7). The main source of soil nitrogen of sampling points 5 (cotton stubble), 7 (wheat stubble) and 43 (wheat stubble) were animal manure in April. Samples 2, 43, 44, and 322 (wheat stubble) in December and 40, 43, and 47 (wheat stubble) in July had similar N sources. This situation is thought to be a result of animal grazing in these fields after harvest. Increased $\delta^{15}N$ values in soils with the application of animal manure have been reported previously (Wagner, 1991). Bol et al. (2005) reported that except for animal manure based on alfalfa hay diets, manure $\delta^{15}N$ value averaged 5.8‰. The variations of $\delta^{15}N$ values with respect to sampling points in three different periods, under different land use types and soil management techniques, are presented in Figure 7. Soil $\delta^{15}N$ value is generally higher in December than those in April. The reason for high $\delta^{15}N$ values in December was probably decomposing plant residues while low values in April were due to the use of excess mineral fertilizers for cotton, maize, tomato, pepper, sunflower, and wheat.

The $\delta^{15}N$ values of the soil samples taken from the north part of the area (dark sections) were $> 10\text{‰}$ (Figure 6). Previous research showed that there was a positive and significant relationship between NO_3^- and Cl^- ions in the soil sampled from the same sampling points ($r^2 = 0.85$) (Ozcan and Kavdir, 2005). These values indicate that sewage water may be leaked here since nitrate originating from sewage has an average $\delta^{15}N$ is around + 20‰ and is very different from chemical fertilizers (Figure 8).

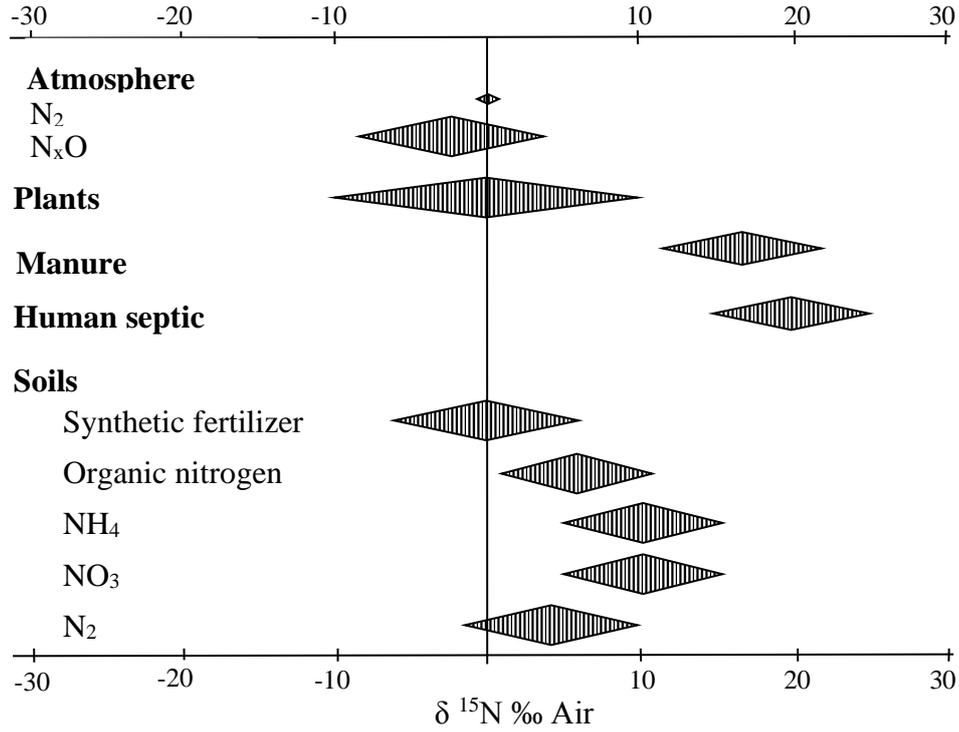


Figure 8. $\delta^{15}\text{N}\%$ values of selected natural sources (Clark and Frits, 1997)
Şekil 8. Seçilen doğal kaynakların $\delta^{15}\text{N}\%$ değerleri (Clark ve Frits, 1997)

Conclusion

According to study results, the highest amount of $\text{NH}_4\text{-N}$ has been observed in the vegetable field ($640 \text{ kg NH}_4\text{-N ha}^{-1}$) where the nitrate content of this soil was $50 \text{ kg NO}_3\text{-N ha}^{-1}$ and SOC concentration was 3.56%. Total nitrogen and inorganic nitrogen analysis in July soil samples showed that a significant part of the inorganic nitrogen was in the form of ammonium in the region while the inorganic N amount varied according to soil texture, land use, and soil management techniques. Pasture lands had the greatest SOC contents in all land use types while most of the SOC values were between 1-2% in the area. The C:N ratio of soils was higher in December due to the incorporation of carbon-rich residues into the soil after harvest. The reason for the low C:N ratio in April and July was the application of inorganic N fertilizers in spring and organic matter mineralization in summer. The $\delta^{15}\text{N}$ values of the soils were generally between 5 and 10‰ indicating that nitrogen was derived from organic materials such as plant residues, microbial biomass nitrogen, and organic fertilizers. Soil $\delta^{15}\text{N}$ value was generally higher in December probably due to decomposing plant residues while low values in April indicate the use of excessive mineral N fertilizers.

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Authors contribution statement

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declare that there is no conflict of interest between them.

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