# Effect of Land Use on Abundance and Diversity of Nematode Destroying Fungi and Soil Nematodes in Embu County, Kenya

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# Abstract

Belowground biodiversity is one of the key indicators of the sustainability of a land use. This study sought to document the occurrence and diversity of nematode destroying fungi in an area characterized by small scale agriculture in Embu County, Kenya. The study area was divided into six main land use types namely; coffee, fallow, forest, maize/bean intercrop, napier and tea. Nematode destroying fungi were extracted from five soil samples from each of the land uses. A total of 161 isolates of nematode destroying fungi belonging to nine genera and 19 species were isolated and identified. The genera represented were Arthrobotrys, Dactyllela, Dactylaria, Harposporium, Monacrosporium, Myzocytium, Nematoctonous, Paecilomyces and Stylophage. The occurrence of nematode destroying fungi was significantly (P=0.02519) affected by the land use types in the study area. With the exception of Arthrobotrys superb, Dactyllela haptospora, Dactyllela reticulate, Harposporium anguillulae and Monacrosporium ellipsoporum all the other 19 isolated species were not affected by land use types. The highest total mean occurrence of nematode destroying fungi was recorded in maize/bean, followed by nappier, coffee, forest, fallow and tea, in that order. The respective frequency of detection of the fungal species was 7.6, 7.2, 5.0, 4.0 and 3.6. The Shannon index of diversity was highest and lowest at 1.971 and 1.177 in the land under maize/bean intercrop and tea, respectively. The species richness was higher in napier, followed by maize bean, coffee, forest and least in tea. Out of the fungi that were isolated, the highest proportion of 23% was from the maize/bean intercrop, while the least 11% was from land under ea. Arthrobotrys oligospora was the most frequently isolated species with a frequency of 24% while Nematoctonus pachysporus had the least frequency of occurrence of 1%. It can be concluded that land use influences the diversity of nematode destroying fungi and nematodes in the soil. More work is needed to determine the efficacy of these indigenous isolates on plant parasitic nematodes.

Keywords: Arthrobotrys oligospora, biological control, plant parasitic nematodes, soil biodiversity

# 1. Introduction

Soil biodiversity refers to the variety of life below the ground. Soil is the home to billions of organisms which range from the microbes like the fungi and bacteria to macrofauna like the termites and earthworms. The other main component of the soil biodiversity is the mesofauna which is represented by nematodes, acari and collembola (Bardgett, 2005). These organisms act individually or interact with one another to provide the soil with the self-perpetuating characteristic of all the ecosystems.

Soil biodiversity has been regarded as the pillar against which the above ground biodiversity rests. It means therefore that plants and animal life including human is directly affected by soil biodiversity. The soil biological activity helps in the maintenance of relatively open soil structure as well as facilitating decomposition and the transportation and transformation of soil nutrients. It also regulates soil pests and diseases and also confers stability to stress and disturbance although the mechanism is not yet fully understood (Brussaard et al., 2007). In short, soil biodiversity is very important to agriculture. Human activities are known to affect the occurrence and diversity of soil biodiversity. For example, the conversion of undisturbed land uses like the forests into agro ecosystems has been reported to impact on the diversity of earthworms (Fragoso et al., 1997). Modern agricultural systems that are characterized by heavy reliance on external inputs like chemical fertilizers and pesticides raises fear on sustainability of such systems (Altieri, 1999).

The study area was located around Irangi forest and its environs (in the Mount Kenya region) within Embu County. It is bounded by longitudes 37° 18' East and Latitudes 0° S and 0° 28' S. The site was selected because it offered a

land use intensification gradient as well as being a mega biodiversity area in Kenya. It is characterized with specific land uses along the altitude gradient. The highest point of the study area is covered by indigenous forest which contains some of the local tree species including *Trichilia emelica* (Meliaceae), *Podocarpus falcalus* (Podocarpaceae) and *Cordia africana* (Boraginaceae) among others. In the cultivated upper cool areas, tea is the main crop. Other agricultural activities include dairy, napier cultivation, passion fruits, potatoes and vegetables. Down the altitude gradient from the tea zone, coffee is grown as a cash crop. Agro-forestry is also highly practiced here together with dairy, nappier, maize/bean with some areas kept fallow. This zone is immediately followed by the maize/bean zone. In this zone, maize, beans, horticultural crops like potato, tomatoes and peas are grown. Other crops like banana and coffee are also grown in this zone Including the dairy, nappier and patches of fallow land.. The three main zones; the tea, coffee and maize/bean receive different types and quantities of agricultural inputs like fertilizers and manure at different times.

Land use intensification in terms of application of organic fertilizers and frequent land tillage/utilization has been reported to reduce soil biodiversity for example nematode community (Kimenju et al., 2009). Soil borne fungal pathogens have also been reported to increase with increased soil disturbance (Maina et al., 2009). Other soil organism like Acari, collebolla, entomopathogenic nematodes and Bacillus were all reported to be affected by land use type (Maribie et al., 2011; Muturi et al., 2009; Kawaka et al., 2011; Wepukhulu et al., 2011).

Plant parasitic nematodes have continued to cause menace in the farms despite the many management strategies proposed and employed, of which application of chemical nematicides has been the most effective and efficient. Unfortunately, these chemicals have been banned due to their negative environmental impacts; human health, non-selective nature and persistence in the soil. There is therefore continued effort to look for alternatives ways to manage plant parasitic nematodes. The utilization of nematode destroying fungi has been proposed. Nematode destroying fungi are a group of soil fungi that are natural enemies of plant parasitic nematodes. They have received a lot of attention for their candidature as biological control agents of plant parasitic nematodes. In nature, they capture and kill nematode destroying fungi in the soil using specialized structures like the adhesive hyphae, constricting rings and adhesive conidia. After the capture, a nematode is completely consumed by the fungi within nine hours (Wachira et al., 2008).

The occurrence and diversity of these nematodes in Kenya have not been fully investigated. Some of the areas where they have been reported include, Taita Taveta, Kabete and Maragua. It is important to document them and seek to develop the most potent isolates for the management of plant parasitic nematodes in agricultural systems. This study therefore was to document the occurrence and diversity of nematode destroying fungi in Embu with the aim of building on the inventory of soil biodiversity in the study area.

#### 2. Materials and Methods

2.1 Description of the Study Area and Soil Sampling



Figure 1. Schematic representation of the intensive sampling in Embu

The study area was stratified into four main zones, namely the lower zone, which was dominated by maize/bean, the middle zone which was mainly under coffee, the upper zone which was on tea and finally the upper most zones under the natural forest. Nappier and fallow land uses were found mainly within the lower, middle and upper land uses. All the sampling sites were demarcated using a Geographical Positioning System (GPS) (Garmin E Trex model) except those in the natural forest due to the forest canopy obstruction.

From each land use type, a total of five farms were identified for intensive sampling through a participatory approach involving the agricultural officers and the farmers. From each farm, a central position was

determined and marked with a GPS. From the center, four diagonals of six meters long were drawn while the soil sampling was done at the three and six meter lengths and the center (Figure 1).

A total of sixteen soil samples were collected at the depth of 0 - 20 cm using a 7 cm diameters soil auger from each farm. All the collected soil samples from one farm were homogenized to make one composite sample from which 500 g of soil was sampled, placed in a plastic bag, labeled and placed in a cool box awaiting transport to the laboratory. The soil auger was wiped with a cotton wool dipped in 75% ethanol after sampling from each farm. The soil samples were transported to the University of Nairobi Mycology laboratory where they were partitioned into three portions, one for soil chemical (nitrogen, phosphorus pottasium, carbon and pH) analysis, another for nematode destroying fungi isolation and the third for nematodes community extraction.

### 2.2 Soil Characterization

The collected soil samples were characterized for nitrogen, phosphorous, carbon, potassium and pH. Total organic carbon was estimated through calorimetric method where all organic carbon in the soil sample was oxidized by acidifying dichromate at 150°C for 30 minutes to ensure complete oxidation. Barium chloride was added to the cool digests. After mixing thoroughly, digests were allowed to stand overnight. The carbon concentration was read on the spectrophotometer at 600 nm (Anderson & Ingram, 1993). Kjeldahl method was used to get the total nitrogen from the soil. Soil samples were digested with concentrated sulphuric acid containing potassium sulphate, selenium and copper sulphate hydrated at approximately 350°C. Total nitrogen was then determined calorimetrically on a flow analyzer (Hinga et al., 1980; Keeny & Nelson, 1982). Soil pH was determined with a pH meter using a soil suspension of 1:1 (w/v) soil. To get the amount of extractable phosporus in the soil, Olsen method was used (Watanabe & Olsen, 1965; Hinga et al., 1980).

# 2.3 Isolation of Nematode Destroying Fungi

One gram of soil from each soil sample was transferred to a previously prepared sterile solid media in a petridish and spread. A suspension of approximately 500 juveniles of *Meladogyne incognita* was added as bait in each sample and incubated at room temperature. Observations on fungal growth were conducted every week after the third week of incubation for three weeks. Observations on dead nematodes and the mycelia growth in the petridish were conducted under dissecting microscope and then under compound microscope using the low power (x40). Identification was based on type and size of conidia, the habit of the conidiophore and the type of nematode destruction structure.

#### 2.4 Extraction of Nematodes

In the laboratory, the soil samples were thoroughly mixed, passed through a 4 mm sieve into a holding pan and partitioned for nematode analysis and storage. Nematodes were extracted from 200 cm<sup>3</sup> soil sub-sample using the centrifugal-floatation method as described by Jenkins (1964). The sample was suspended in 4-5 litres of tap-water in a 20 litre-bucket and stirred by hand for 10 seconds to release the nematodes from the soil. The slurry of water was then decanted through a 2 mm sieve into a second bucket and the filtrate was then run through a series of fine aperture sieves namely; 250  $\mu$ m, 150  $\mu$ m and then 38  $\mu$ m. The residue collected on each sieve was backwashed and concentrated to form a 30 ml volume that was transferred into 50 ml-centrifuge tube. This suspension was spinned twice, first at 1750 rpm for 7 min and then supernatant at 1750 rpm for 3 minutes for each individual sample. The supernatant formed after the second spin was poured into excess water and concentrated using the 38  $\mu$ m aperture sieve to make a 3 ml nematode suspension. Nematodes in each sample were then killed and fixed as described by Hooper (1986). The fixed specimens were refrigerated at 4°C for settling, enumerated and then identified. All nematodes enumerated were then grouped into trophic levels; herbivore, fungivore, bacteriovore, omnivore and predator as described by Yeates et al. (1993) and M. Bongers and T. Bongers (1998).

# 3. Results

# 3.1 Soil Characteristics

It was observed that nitrogen, phosphorus, pottasium (parts per million), percentage carbon and pH levels varied among land use systems in the study area. The soil pH levels varied from 3.5 to 4.2 with the highest pH being recorded in land under fallow with a record of 4.19 while the least pH (3.54) was recorded in the natural forest. Carbon was highest in fallow, natural forests, tea, nappier, coffee, and then maize bean in that decreasing order, while total nitrogen was highest in fallow (0.74) and least in coffee (0.32) land use. Percentage carbon was highest in fallow (5.81) and least in coffee. Potassium was highest in tea, then coffee, nappier, natural forests, maize bean and the least in fallow with records of 0.38, 0.33, 0.31, 0.28, 0.27 and 0.19 respectively. Phosphorus levels varied from 21.13 in natural forest to the least record of 10.83 in coffee land uses (Table 1).

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Land use	pН	N%	С%	P ppm	K cmol/kg
Coffee	4.03	0.32	3.43	10.83	0.33
Fallow	4.19	0.74	5.81	16.63	0.19
Maize/bean	3.88	0.37	3.70	16.13	0.27
Napier	4.14	0.33	3.87	14.75	0.31
Natural forest	3.54	0.56	5.43	21.13	0.28
Tea	3.86	0.44	4.69	14.60	0.38

Table 1. Mean chemical characteristics of soil under varying land use types in Embu

#### 3.2 Nematode Destroying Fungi

From this study, nematode destroying fungi were recorded in all the areas under the study. A total of 161 isolates of nematode destroying fungi distributed into nine genera and 19 species were isolated and identified. The genera included; *Arthrobotrys, Dactyllela, Dactylaria, Harposporium, Monacrosporium, Myzocytium, Nematoctonous, Paecilomyces and Stylophage.* The results also indicated that the occurrence of nematode destroying fungi was significantly (P = 0.02083) affected by land use type with the highest means of total occurrence being recorded in the maize/bean (7.4) land uses while the least was in tea (7.2) land use (Figure 2).



Figure 2. Mean total occurrence of nematode destroying fungi in various land use types in Embu

Nematode destroying fungi were most diverse in land under maize/bean with a Shannon index of 1.971 while the tea was the least diverse with a Shannon index of 1.177. Maize/bean and nappier were the richest in terms of nematode destroying fungi with mean richness of 7.4 and 7.2 respectively. Twenty three percent (23%) of all the fungal isolates were obtained from maize/bean land use followed by nappier with 22.4 %, then natural forest and coffee with 15.2 each, fallow with 12.4 and the least was tea with only 11.2 % (Table 2).

Land use	Mean richness	Mean shannon	Total abundance	Percentage occurrence	
Coffee	5	1.436	25	15.52	
Fallow	4	1.326	20	12.42	
Forest	5	1.601	25	15.52	
Maize/bean	7.4	1.971	37	22.98	
Napier	7.2	1.898	36	22.36	
Tea	3.6	1.177	18	11.2	

Table 2. Occurrence of nematode destroying fungi per land use in Embu

The most frequently encountered species was *Arthrobotrys oligospora* which had a frequency of occurrence of 14.9% followed by *Arthrobotrys dactyloides* with a frequency of 12.4 %. The least frequently isolated fungal isolate was *Nematochtonus pachysporus* whose frequency of isolation was 0.62%. Only five of the 19 nematode destroying fungi isolates, namely *Arthrobotrys superba*, *Dactyllela haptospora*, *Dactyllela reticulate*, *Harposporium anguillulae and Monacrosporium ellipsoporum* were significantly (p> 0.05) affected by the land use type (Table 3). From the species cumulative curve, it was evident that all the possible species of nematode destroying fungi were isolated within the sample size (Figure 3)

Table 3. Effect of land use on the total nematode destroying fungi in Embu

Species	Coffee	Fallow	Forest	Maize/beans	Napier	Tea	P value
A. candida	0	0	0.4	0.2	0.2	0	0.3653
A. dactyloides	0.6	0.4	0.6	1	1	0.4	0.1379
A. robusta	0.2	0	0.4	0	0.4	0	0.2428
A.superba	0	0	0	0.2	0.6	0	0.01580
A.oligospora	0.8	0.8	0.6	1	0.8	0.8	0.8187
D. cionopaga	0.2	0	0	0	0.2	0	0.5606
D. haptospora	0	0	0	0.4	0.4	0	0.09598
D.leptosporus	0	0	0	0.2	0.4	0	0.1778
D.bronchopaga	0	0.2	0	0.2	0	0	0.5606
D.candida	0.2	0	0	0.2	0.4	0	0.3653
D.reticulata	0	0	0	0.4	0.4	0	0.09598
H.anguillulae	0.4	0.2	0.6	1	0.2	0	0.01069
M.cionopagum	0.2	0.6	0.2	0.4	0.4	0.4	0.8267
M. ellipsoporum	0.4	0.6	0.4	0	0	0	0.07115
M.coniospora	0.2	0.2	0.4	0.4	0.2	0	0.7246
M.asterospermum	0.2	0	0	0.2	0.2	0.4	0.5904
Myzocytium.spp	0	0	0.2	0	0	0.4	0.1778
N. concurrens	0	0	0.2	0.2	0.4	0	0.3653
N. leiosporus	0.4	0	0	0	0	0.2	0.1778
N. pachysporus	0	0	0	0	0.2	0	0.4389
N.tylosporus	0	0	0.2	0	0.2	0.4	0.3653
N.geogenius	0.2	0.4	0.3	0.6	0	0	0.1877
P.lilacinus	0.6	0.4	0.6	0.8	0.6	0.4	0.844
S. grandis	0.4	0.2	0	0	0	0.2	0.3653

#### KEY:

A .oligospora	Arthrobotrys oligospora
A. candida	Arthrobotrys candida
A.dactyloides	Arthrobotrys dactyloides
D. bronchopaga	Dactylella bronchopaga
D. candida	Dactylaria candida
D. cionopaga	Dactylella cionopaga
D. haptospora	Dactylaria haptospora
D. leptosporus	Dactvlella leptosporus

H.anguillulae P.lilacinus S. grandis N. pachysporus M.cionapagum M.elliposporium A.superba D. reticulata Harposporium anguillulae Paecilomyces lilacinus Stylophage grandis Nematoctonus pachysporus Monacrosporium cionapagum Monacrosporium ellipsosporum Arthrobotrys superba Dactylella reticulate



Figure 3. Species cumulative curve for nematode destroying fungi in Embu, Kenya

The occurrence and diversity of nematode destroying fungi was affected by addition of organic matter and continuous tillage as indicated by the principal component analysis. More than 45% of the nematode destroying fungi were isolated from soil under nappier and maize/bean which were grouped together by the factor 1Tea, coffee, fallow and natural forest were grouped together by factor 1 as well. The second factor grouped tea and nappier together while maize/bean, natural forest, fallow and coffee were clustered together.

#### 3.3 Nematode Community

The occurrence of soil nematode was significantly (P=0.05) affected by land use types. Maize/bean intercrop had the highest record of nematodes followed by napier, natural forest, fallow, and coffee with mean abundance of 145, 141, 140.8, 133.2,81 and 71.8, respectively. Species eveness was highest in land under nappier while it was least under tea. The diversity index was highest and lowest at 1.345 and 0.687 in land under napier and tea, respectively (Table 4).

Land use	Mean richness	Mean abundance	Mean shannon	Mean evenness
Coffee	3.4	81.0	0.976	0.813
Fallow	4.4	133.2	1.136	0.729
Maize/bean	4.8	145.0	1.232	0.727
Napier	4.6	141.0	1.345	0.845
Natural forest	4.4	140.8	1.095	0.693
Tea	3.2	71.8	0.687	0.657

Table 4. Species richness, abundance, Shannon and evenness of nematodes in land under varying uses in Embu County

All the extracted soil nematodes were grouped into four trophic levels, bacteriovores, fungivores, herbivores, omnivores and predator. Of the four groups, the fungivores, herbivores and omnivores were significantly (P > 0.05) affected by land use types unlike the predators and the bacteriovores which were not (Table 5).

Trophic group	Coffee	Fallow	Maize bean	Napier	Natural Forest	Tea	P -value
Bacteriovore	26.4	44.4	39.0	42.8	27.8	44.8	0.7217
Fungivores	0.6	12.0	21.8	28.6	7.8	0.2	0.0001159
Herbivores	22.0	11.2	66.2	19.0	17.4	9.0	0.0002442
Omnivores	30.2	58.2	7.4	43.2	75.4	17.0	1.137 x 10 <sup>-05</sup>
Predator	1.8	7.4	10.6	7.4	12.4	0.8	0.502

Table 5. Effect of land use on nematode trophic levels in Embu

The species cumulative curve for the nematodes indicated that 10 samples were sufficient to estimate all the trophic levels of nematodes in the study area (Figure 4).



Figure 4. Species cumulative curve for the nematode trophic levels in Embu

The Renyi diversity profiles showed that napier was the most diverse land use followed by coffee, then fallow, maize bean, natural forest and the least land use was tea (Figure 5).





# 4. Discussion

From the study, it was evident that soil characteristics differed with land uses. Land uses with least disturbance (fallow and natural forest) had the highest carbon of more than 5% compared to the cultivated land uses. This high amount of carbon could be attributed to the fact that high amount of organic material from plants fall and decompose on the soil surface of these land uses. Bedano et al. (2006), noted that soils with plant residues provides readily available food source for soil organisms, moderate the effect of extreme temperature and also prevent moisture loss. This in turn increases soil biodiversity. Contrally to the expectation that the natural forest would have the highest percentage nitrogen because of the high organic matter and least disturbance, this was not the case. However nitrogen and carbon were highest in undisturbed land uses (fallow and natural forest) compared to the disturbed (cultivated) land uses. However the amount of phosphorus was highest in the forest but least in coffee. These results are in agreement with those by Muya et al. (2009). The amount of nitrogen in the cultivated land use was low because it mainly depended on the type and amount of fertilizers applied by the farmers. Application of fertilizers to coffee is not common in the area compared to other crops that seem to take priority. This could be attributed to the low prices of coffee hence lack of morale by the farmers. This finding is consistent with Mutsotso et al. (2011) who noted that the low prices of coffee in Embu had led to abandonment of the crop.

Nematode destroying fungi occurred in all the sampled land uses in Embu. The result confirms the ubiquitous nature of the nematode destroying fungi in the soil in agreement with other studies on occurrence and diversity of nematode destroying fungi (Gray, 1985; Wachira et al., 2008). The nematode destroying fungi were more in the cultivated land compared to the uncultivated. This could be attributed to the regular turning of the soil and also by the high diversity of crops hence high biological activity. In exception to this, soils under tea receive regular quantities of fertilizers, are hardly cultivated and are always covered by the tea canopy. These factors might have contributed to the low population of nematode destroying fungi. Application of chemical fertilizers has been attributed to low biological activity reflected low microbial counts and microbial biomass carbon of the soil (Nakhro & Dkhar, 2010). Regular turning of the soil which is more common in maize/bean land uses than in tea land uses was found to be a factor affecting distribution of nematode destroying fungi hence increasing their frequency of isolation (Wachira et al., 2009). Contrally to this, although napier land used do not receive regular application of fertilizer nor turning of the soil, they were found to host high numbers of nematode destroying fungi. This could be attributed to the regular application of farmyard manure and harvesting. The manure increases biological activity while the harvesting opens the soil cover. In a study on mycorhizal fungi in Embu, Jefwa et al., (2009) reported that napier land uses had the highest diversity of mycorrhizal spores compared to other land uses. Taking the natural forest as the benchmark, fallow, tea, and coffee land uses have lower population of nematode destroying fungi. This could be attributed to low plants diversity compared to the natural forests (Maitima et al., 2009). Plants diversity could affect the occurrence and diversity of nematode destroying fungi in the soil. This is because; the belowground biodiversity is thought to affect the associated aboveground biodiversity. Kawaka et al. 2011 noted that conversion of forest to cultivate a monocrop reduced the chances of isolating entomopathogenic nematodes. In their argument, they noted that single vegetation is usually characterized with fewer pests which would again affect the host-parasite relationship. This therefore might have explained the low population of nematode destroying fungi in tea and coffee land uses. However, this study contradicts some studies on soil biodiversity which found that high population are found in the forests and fallow land uses because of high availability of carbon and nitrogen (Bedano et al., 2006; Maribie et al., 2011). In contrast, the population of nematode destroying fungi is dependent mostly on crops diversity and more so in increased agricultural intensification. It is also highly proposed that this group of fungi will be influenced by the population and diversity of soil nematodes (Wachira & Okoth, 2009).

The soil nematodes were also affected by the land use types. The nematodes were isolated in all the land uses meaning that they are universal soil organisms. This information is important for the farmers so that they be aware of the presence of nematodes in their farms. Like in the nematode destroying fungi, the highest population was recorded in the maize/bean and the napier land uses. This particular finding is important because it shows that the population of nematodes and nematode destroying fungi follow one other. Probably the populations of the nematode destroying fungi follow the soil nematodes for food. Although no direct relationships have been observed for particular genera, Wachira et al. (2009) reported an inverse relationship between nematode destroying fungi and plant parasitic nematodes. The nematodes were least in the tea land use. This indicates that the tea land use has very low biological activity which, coupled with the monocrop husbandry are known to reduce biological diversity (Kawaka et al., 2011).

In conclusion, the results from this study show that soil chemical properties are affected by anthropogenic activities. They in turn affect the occurrence of the above ground biodiversity and concomitantly the soil

biodiversity but are not them main factors in determining the occurrence of nematode destroying fungi. It is also clear that mono-crop reduces biological activity and the associated soil organisms. From this study, it is also evident the study site is rich in nematode destroying fungi and adds to the list of inventorised soil biodiversity in the area. The study also revealed that the population of nematode destroying fungi fallows that of the nematodes. In particular, the occurrence of high population of herbivore nematodes in the maize/bean land use where there is high population of nematode destroying fungi is quite surprising. It would be expected that high population of nematode destroying fungi isolates (161), it does not find a direct conclusion that nematode destroying fungi are reducing the population of herbivore nematodes. This creates a room for further investigation on correlations of the two populations and especially on relationship between specific nematode destroying fungi genera and plant parasitic nematodes.

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